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Research Article

Exploring benthic diatom diversity in the West Antarctic Peninsula: insights from a morphological and molecular approach

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Abstract

Polar regions are among the most extreme habitats on Earth. However, diatom biodiversity in those regions is much more extensive and ecologically diverse than previously thought. The objective of this study was to add knowledge to benthic diatom biodiversity in Western Antarctic coastal zones via identification by means of morphology, DNA metabarcoding and cultured isolates. In addition, a taxonomically validated reference library for Antarctic benthic diatoms was established with comprehensive information on habitat, morphology and DNA barcodes (*rbc*L and 18SV4). Benthic samples from marine, brackish and freshwater habitats were taken at the Antarctic Peninsula. A total of 162 clonal cultures were established, resulting in the identification of 60 taxa. The combination of total morphological richness of 174 taxa, including the clones, with an additional 73 taxa just assigned by metabarcoding resulted in 247 infrageneric taxa. Of those taxa, 33 were retrieved by all three methods and 111 only by morphology. The barcode reference library of Antarctic species with the new references obtained through culturing allowed the assignment of 47 taxa in the metabarcoding analyses, which would have been left unassigned because no matching reference sequences were available before. Non–metric multidimensional scaling analyses of morphological as well as molecular data showed a clear separation of diatom communities according to water and substratum types. Many species, especially marine taxa, still have no record in reference databases. This highlights the need for a more comprehensive reference library to further improve routine diatom metabarcoding. Overall, a combination of morphological and molecular methods, along with culturing, provides complementary information on the biodiversity of benthic diatoms in the region.

Key words: Antarctic Peninsula, benthic diatoms, DNA metabarcoding, morphology, *rbc*L, taxonomic reference library, unialgal cultures, 18SV4

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Introduction

The polar regions are among the most extreme environments on Earth. Total darkness in winter is paired with low temperatures, strong winds and heavy snow cover. In contrast, permanent light and higher temperatures in summer

result in ice and snow melt (Pavlov et al. 2019). Marine biota living in those regions must deal with extreme seasonality of light, temperature, salinity and sea ice (Zacher et al. 2009). In contrast to this harsh environment, biodiversity in polar regions is much more extensive, ecologically diverse, and biogeographically structured than previously thought and the prevalence of such conditions for millions of years has led to the evolution of a truly unique flora and fauna (Griffiths 2010; Chown et al. 2015; Danis et al. 2020).

An ecologically particularly important group of eukaryotic microorganisms in Antarctic shallow water coastal zones are benthic diatoms living on top of or associated with sediments, rocks or sea ice. Their benthic assemblage exerts multiple important functions as primary producers, providing a major food source for a diverse range of organisms such as bacteria by excretion of soluble organic matter, benthic protozoans as well as metazoans (Cahoon 1999), including mesograzers such as amphipodes and gastropodes (Zacher et al. 2007; Campana et al. 2008; Aumack et al. 2017; Amsler et al. 2019). Furthermore, diatoms influence elemental fluxes at the sediment–water interface (Risgaard–Petersen et al. 1994) and stabilize the sediment surface by excretion of sticky extracellular polymeric substances (de Brouwer et al. 2005). Due to their abundance, marine planktonic diatoms account for up to one fifth of the global photosynthetic carbon fixation (Falkowski et al. 2000).

Numerous recent studies indicate that microorganisms display a distinct biogeography, which is also strongly supported by evidence from different freshwater and soil diatoms (Vanormelingen et al. 2008; Abarca et al. 2014; Pinseel et al. 2020). Freshwater benthic diatoms in Antarctica have been intensively studied e.g. Van de Vijver et al. (2002); Kopalová et al. (2015); Sterken et al. (2015); Zidarova et al. (2016a, b); Van de Vijver et al. (2018) and revisions of freshwater Antarctic and sub–Antarctic diatom floras point to a strong regionalization (Vyverman et al. 2010; Verleyen et al. 2021). Despite their crucial role, information about the biodiversity of Antarctic marine benthic diatoms is scarce and only a few studies exploring their biodiversity exist (Klöser 1998; Al-Handal and Wulff 2008a, b; Campana 2018; Al-Handal et al. 2022; Zidarova et al. 2022).

DNA metabarcoding has emerged as an alternative to light microscope-based identifications (LM) as it provides a faster and cheaper way of identifying species in an environmental sample because the morphological identification and counting of diatoms species in LM is time–consuming and demands extensive expertise since diatom taxonomy is constantly evolving. (Kermarrec et al. 2014; Zimmermann et al. 2015). This approach has been used to investigate freshwater diatom biodiversity (Rimet et al. 2018b; Mora et al. 2019) and has been applied to some extent to marine environments (Malviya et al. 2016; Piredda et al. 2018; Pérez-Burillo et al. 2022). Benthic diatoms are commonly used as bioindicators to monitor water quality because of their rapid response to environmental pressures and their omnipresence (Rimet and Bouchez 2012; Desrosiers et al. 2013). DNA metabarcoding based on benthic diatoms has been utilized to monitor community changes and assessing the biological status of a water body (Vasselon et al. 2017; Bailet et al. 2019; Mortágua et al. 2019; Kelly et al. 2020; Pérez-Burillo et al. 2020) and a taxonomy-free biomonitoring approach has emerged that allows the computing of a molecular index directly without any reference to morphotaxonomy to overcome the limitatios of the reference

databases and the lack of phylogenetic resolution (Apothéloz-Perret-Gentil et al. 2017; Tapolczai et al. 2019a, b, Gregersen et al. 2023).

For a reliable identification, an unambiguous link between geno– and phenotype is crucial. Therefore, a comprehensive taxonomic reference library is required where molecular and morphological data are tied together with a taxonomic name (Zimmermann et al. 2014; Stachura-Suchoples et al. 2015). For diatoms, clone cultures need to be established which offer sufficient material for sequencing as well as for identification by light and electron microscopy. Finally, all reference sequences should be linked to diatom voucher specimens deposited in a herbarium in order to offer a complete chain of evidence back to the formal taxonomic literature.

The objective of this study was to add knowledge to the biodiversity of marine benthic diatoms in Western Antarctic shallow water coastal zone environments. In addition, some brackish and freshwater environments connected to the marine realm were explored. Benthic diatom biodiversity in communities sampled in Potter Cove, King George Island/ Isla 25 de Mayo, West Antarctic Peninsula were identified by the means of morphological and molecular methods to assess the status of their taxonomic coverage in Antarctic regions. To compare the performance of morphology and metabarcoding in the identification and quantification of diatom abundances, our objective was to compare the number of taxa retrieved by both analysis of environmental samples. A further goal was to create a regional vouchered barcode reference library with the help of clone cultures with comprehensive information on habitat, morphology and DNA barcodes (*rbc*L and 18SV4). This taxonomic reference library was utilized for DNA metabarcoding to access the concealed biodiversity beyond the limits of morphological and cultivating methods. Generating the thus far most extensive biodiversity dataset on Antarctic marine benthic diatoms provides a reference to monitor community changes to predict the potential impact of climate change on the coastal ecosystems of this region.

Methods

Study area and sampling collection

Epipsammic and epilithic samples from marine, brackish and freshwater habitats were taken in Austral summer 2020 at Potter Cove, a shallow coastal bay at King George Island/ Isla 25 de Mayo, West Antarctic Peninsula (Fig. 1). Potter Cove combines zones of glacier fronts and rocky shores as well as extensive soft bottom areas and thereby providing diverse habitats for benthic diatoms (Klöser 1998).

In total 39 samples were taken (Table 1, Fig. 1). At eight of the locations freshwater samples were taken from glacial run–off water or drinking water reservoirs. At 17 locations the littoral zone was sampled, and additional 14 marine locations were sampled by scuba diving reaching down to a water depth of 20 m (Table 1). A map of the sampling points was generated with the software QGIS 2.18 (QGIS Development Team 2021).

At each sample location a composite sample of 60 ml was taken along a transect of approximately 10 m. At sample locations with rocky substrate the biofilm of three to four stones along the transect was scratched with a knife.

Table 1. Sample sites with information on the location, georeference, altitude, collector, water type, substrate type and voucher at the BGBM.

Sample ID	Sampling date	Location	GPS coordinates	Altitude	Collector	Water type	Substrate type	Voucher at BGBM
D283	28.01.2020	Coastal zone at Peñón 1	62.245938°S, 58.681731°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021363
D284	28.01.2020	Lighthouse Melting Pond	62.240866°S, 58.677563°W	28 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021364
D285	29.01.2020	IT Resevoire	62.237876°S, 58.662233°W	12 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021365
D286	29.01.2020	Drinking water pond at Carlini station	62.238091°S, 58.657689°W	23 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021366
D288	29.01.2020	Coastal zone at Peñón 0	62.241809°S, 58.681931°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021367
D289	30.01.2020	Coastal zone at island A7	62.234665°S, 58.664624°W	10 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021368
D290	30.01.2020	Coastal zone at island A7	62.234665°S, 58.664624°W	10 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021369
D292	30.01.2020	Coastal zone at island A7	62.234665°S, 58.664624°W	10 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021370
D293	30.01.2020	Coastal zone at island A7	62.234665°S, 58.664624°W	10 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021371
D294	30.01.2020	Coastal zone east of Carlini station	62.235314°S, 58.656489°W	0 m	J. Zimmermann	brackish water	epipsammic biofilm	B 50 0021372
D295	30.01.2020	Coastal zone east of Carlini station	62.235771°S, 58.658364°W	0 m	J. Zimmermann	brackish water	epipsammic biofilm	B 50 0021373
D296	31.01.2020	Coastal zone at island A4	62.229219°S, 58.663369°W	15 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021374
D297	31.01.2020	Coastal zone at island A4	62.229219°S, 58.663369°W	15 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	episammic biofilm	B 50 0021375
D299	01.02.2020	Glacier meltwater run-off in Tres Hermanos area	62.251939°S, 58.652703°W	60 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021376
D300	01.02.2020	Drinking Water Reservoire	62.237861°S, 58.662250°W	51 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021377
D301	04.02.2020	Coastal zone at island A4	62.229219°S, 58.663369°W	5 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	biofilm from stones	B 50 0021378
D302	04.02.2020	Coastal zone at island A4	62.229219°S, 58.663369°W	5 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021379
D303	04.02.2020	Glacier meltwater run-off Fourcade	62.236639°S, 58.647028°W	10–15 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021380
D304	04.02.2020	Glacier meltwater run-off Fourcade	62.236639°S, 58.647028°W	10–15 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021381
D305	05.02.2020	Coastal zone at island A4	62.229219°S, 58.663369°W	20 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021382
D306	06.02.2020	Coastal zone at Punta Elefante	62.237353°S, 58.679569°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021383
D307	07.02.2020	Coastal zone at Peñón 1	62.247261°S, 58.680051°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021384
D308	07.02.2020	Coastal zone at Peñón 1	62.247261°S, 58.680051°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021385
D309	07.02.2020	Diver's container at Carlini station	62.237459°S, 58.667529°W	2 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	biofilm from stones	B 50 0021386
D310	07.02.2020	Coastal zone at Peñón de Pesca	62.237906°S, 58.712278°W	5 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	biofilm from stones	B 50 0021387
D311	08.02.2020	Coastal zone at Punta Stranger	62.256388°S, 58.625618°W	2 m	J. Zimmermann	marine	biofilm from stones	B 50 0021388
D312	08.02.2020	Coastal zone at Punta Stranger	62.256296°S, 58.626069°W	2 m	J. Zimmermann	marine	biofilm from stones	B 50 0021389
D313	08.02.2020	Coastal zone at Punta Stranger	62.258227°S, 58.642172°W	1 m	J. Zimmermann	marine	biofilm from stones	B 50 0021390
D314	09.02.2020	Glacier meltwater run-off Refugio Albatros	62.252046°S, 58.659456°W	49 m	J. Zimmermann	freshwater	biofilm from stones	B 50 0021391
D315	09.02.2020	Coastal zone at Peñón 4	62.256107°S, 58.659703°W	2 m	J. Zimmermann	marine	biofilm from stones	B 50 0021392
D316	09.02.2020	Coastal zone at Peñón 2	62.250540°S, 58.675029°W	2 m	J. Zimmermann	marine	biofilm from stones	B 50 0021393
D317	09.02.2020	Coastal zone at Peñón 1	62.247073°S, 58.683764°W	2 m	J. Zimmermann	marine	biofilm from stones	B 50 0021394
D318	10.02.2020	Coastal zone at Peñón 2	62.250704°S, 58.675778°W	1 m	J. Zimmermann	marine	biofilm from stones	B 50 0021395
D319	12.02.2020	Coastal zone at Carlini station	62.236950°S, 58.663583°W	1 m	J. Zimmermann	marine	biofilm from stones	B 50 0021396
D320	13.02.2020	Coastal zone at Punta Stranger	62.256109°S, 58.630578°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021397
D321	13.02.2020	Coastal zone at Punta Stranger- Peñón 4	62.256615°S, 58.641681°W	0 m	J. Zimmermann	marine	biofilm from stones	B 50 0021398
D322	14.02.2020	Coastal zone at island A2	62.227633°S, 58.678734°W	10 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021399
D324	16.02.2020	Coastal zone at island A6	62.223800°S, 58.642639°W	15 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021400
D325	17.02.2020	Coastal zone at island A6	62.223800°S, 58.642639°W	20 m deep	J. Zimmermann, G. L. Campana, Divers Carlini Station	marine	epipsammic biofilm	B 50 0021401

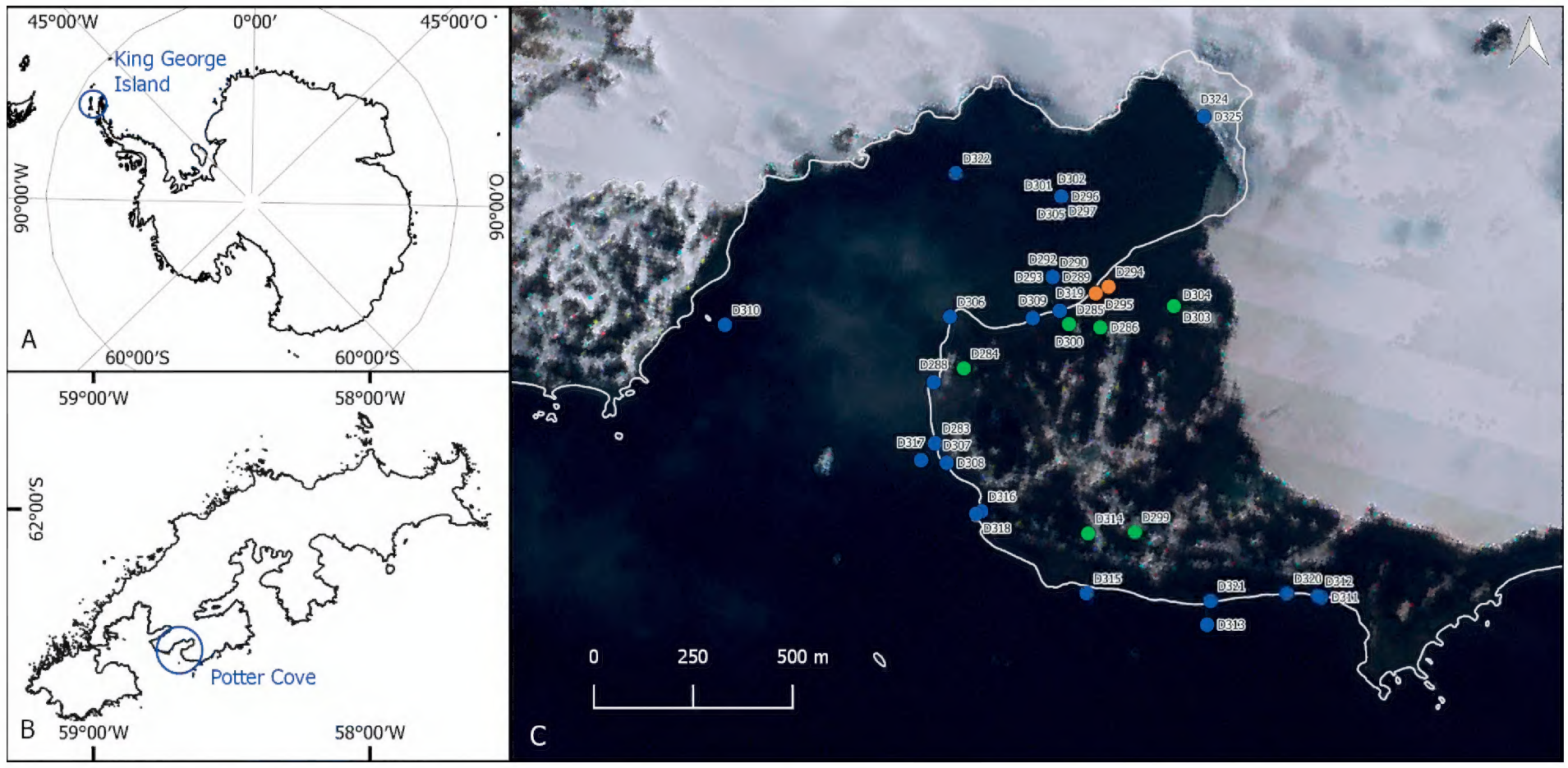

Figure 1. A Map of Antarctica. **B** Map of King George Island/Isla 25 de Mayo. **C** Map of the Potter Cove, with the 39 sample locations. Blue points represent marine sample locations, green points represent freshwater sample locations and orange points represent brackish water locations. Basemap: Landsat Image Mosaic of Antarctica (LIMA).

At locations with soft sediment a sediment corer was used to collect the material of three to four spots along the transect. The top layer of the cores was then sampled with a syringe. The composite samples were homogenized, and divided into 3 subsamples of 20 ml each, which were used for 3 different purposes: 1) fixed in 70% alcohol for morphological identification of the mixed diatom community, 2) stored cooled for the establishment of clone cultures to build the barcode library and 3) fixed in 99% ethanol and frozen for a community analysis via DNA metabarcoding.

Establishment of clonal cultures

Following the procedures outlined in Skibbe et al. (2022), benthic diatoms were isolated from aliquots of environmental samples to establish clonal cultures afterwards. For this purpose, a small subsample of the biofilm was transferred from the collected environmental samples to 5 cm (diameter) Petri dishes filled with liquid culture media. Different media were used for each sample to obtain as many species with different requirements as possible. The cultivation media was prepared with sterile water enriched with one of the following media: f/2 seawater medium (Guillard and Ryther 1962), Alga–Gro medium (Carolina Biological Supply Company) or Walne's medium (Walne 1970) and salted up to a salinity of 34 psu in case of a marine sample and 12 psu for brackish samples. Using an inverted light microscope (100–400× magnification, Olympus) and microcapillary glass pipettes, single cells were transferred into microwell plates containing culture medium. After reaching sufficient densities, isolates were transferred to 5 cm petri dishes. All water samples, isolates and cultures were maintained at 5–7 °C. Illumination was accomplished by white light LEDs under a 16/8 day/night cycle with 15 min dark phases every hour during the day to prevent photo–oxidative stress.

Morphological analysis from environmental samples and clonal cultures

Environmental samples and material harvested from the unialgal cultures were treated with 35% hydrogen peroxide at room temperature to oxidize the organic material and washed with distilled water as described in Mora et al. (2019). To prepare permanent slides for light microscopy analyses, the cleaned material (frustules and valves) was dispersed on cover glasses, dried at room temperature and embedded with the high refraction index mounting medium Naphrax.

Each environmental sample was inspected for their benthic diatom composition using LM. Observations were conducted with a Zeiss Axioplan Microscope equipped with Differential Interference Contrast (DIC) using a Zeiss 100× PlanApochromat objective. Microphotographs were taken with an AXIOCAM MRc camera. To record the occurrence and abundance of each diatom taxon at all sampling sites, at least 400 frustules were counted per sample and the relative abundance of each taxon calculated. All samples were scanned for rare species.

Furthermore, morphological identification of the unialgal cultures were conducted also by LM and extended by scanning electron microscopy (SEM) if appropriate. Therefore, aliquots of cleaned culture material were dried on silicon wafers and mounted on stubs and observed under a Hitachi FE 8010 scanning electron microscope operated at 1.0 kV.

Molecular identification of diatom cultures

Cultured material was first centrifuged, and culture medium was discarded by carefully pipetting. DNA was isolated from the remaining pellet using NucleoSpin Plant II Mini Kit (Macherey–Nagel, Düren, Germany) following product instructions. DNA fragment size and concentrations were evaluated via gel electrophoresis (1.5% agarose gel) and Nanodrop (PeqLab Biotechnology LLC; Erlangen, Germany) respectively. Amplification was conducted by polymerase chain reaction (PCR) after Zimmermann et al. (2011) for the V4 region of 18S. The protein–coding plastid gene *rbc*L was amplified after Abarca et al. (2014) with M13 tailed primers rcbL–iF/*rbc*L–R. PCR products were visualized in a 1.5% agarose gel and cleaned with MSB Spin PCRapace (Invitek Molecular GmbH, Berlin, Germany) following manufacturer instructions. Concentrations of PCR products were measured using Nanodrop (PeqLab Biotechnology) and normalized to >100 ng/µl for sequencing. Sanger sequencing was conducted bidirectionally by Starseq (GENterprise LLC; Mainz, Germany), with the same primers used for the amplifications. The DNA material is stored in the Berlin DNA Bank Network (Gemeinholzer et al. 2011).

DNA metabarcoding

A volume of 2–4 ml of each sample was centrifuged at 4 °C and 11.000 rpm for 5 min. The supernatant was removed and from the remaining pellet the DNA was extracted with the NucleoSpin Soil Kit (Macherey and Nagel) following the manufacturer instructions. Short areas of the hypervariable region V4 of the 18S rRNA gene and the *rbc*L plastid gene were amplified in separated target PCRs. For the 18S V4 region the Nextera primers DIV4for: 5′ – GCGGTAATTCCAGCTCCAATAG–3′ and DIV4rev3: 5′ – CTCTGACAATGGAATACGAATA–3′ were used after Zimmermann et al. (2011) with a modification for 300–bp paired–end sequencing for Illumina

MiSeq following Visco et al. (2015). The *rbc*L marker was amplified using an equimolar mix of the modified versions of the Diat_*rbc*L_708F and R3 primers established by Vasselon et al. (2017). For each sample PCR was once repeated for technical replication. Purification of the samples was performed with 25 ml aliquots of the amplicons with HighPrep PCR Clean–up System (Magbio Genomics). Indexing PCR on the purified samples to ligate a unique combination of tags to the 5' end of the primer, DNA quantitation and Illumia MiSeq v3 sequencing (300 bp paired–end reads) with 600 cycles were conducted at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv) of the Berlin Brandenburg Institute of Advanced Biodiversity Research (BBiB).

Raw demultiplexed reads were deposited at GenBanks Sequence Read Archive and are publicly available under project number PRJNA997374.

Bioinformatic analysis

The BeGenDiv performed demultiplexing of the samples providing two fastq files per sample containing forward reads (R1) and reverse reads (R2) respectively. Primers were removed from the reads with cutadapt (Martin 2011). To process the resulting reads the R package DADA2 was used (Callahan et al. 2016). The quality profile was checked, and reads were truncated consecutively for *rbc*L at R1 to 200 bp and at R2 to 160 bp and for 18SV4 at R1 to 230 bp and at R2 to 170 bp. Truncated reads were filtered using a maximum expected error rate of 2. Hereinafter, amplicon sequence variants (ASVs) were selected based on the error rates model determined by the DADA2 denoising algorithm and paired reads were merged into one sequence. Chimeras were identified and removed from the dataset.

Taxonomic assignment for each barcode was performed using an own established reference library comprising the Diat.barcode library (Rimet et al. 2019), the reference library of the Diatom research group of the Botanic Garden Berlin (5768 taxa for 18SV4 and 5604 taxa for *rbc*L) and the newly generated sequences from Antarctic cultures. In case of unclassified taxa on phylum level, the ASV was checked using the Basic Local Alignment Search Tool (BLAST, Camacho et al. 2009) against NCBI GenBank.

After bioinformatic analyses with DADA2 the R package metabaR was used to identify artefactual sequences like contaminants and tag–jumps (Zinger et al. 2021). The dataset was checked for dysfunctional PCRs based on PCR replicate similarities. Then, reads from replicates were aggregated.

Data analysis

Venn diagrams with eulerr (Larsson 2021) were used to visualize how well morphology (LM of environmental samples and cultures) and DNA metabarcoding were able to identify taxa. Barplot diagrams on genus level were generated for the metabarcoding and morphology data using the R package phyloseq (McMurdie and Holmes 2013). Alpha diversity indices (taxa richness and Shannon diversity index) were calculated with the vegan 2.6 R package (Oksanen et al. 2022). Differences in community structure regarding water types (marine, brackish water and freshwater) and substrate (epipsammic biofilm, biofilm on rocks) between samples based on metabarcoding and morphology at the ASV– and species level respectively were calculated by a Bray–Curtis

dissimilarity measure using phyloseq and visualized through non–metric multidimensional scaling (NMDS) ordination. Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the statistically significant differences in diatom community composition regarding water and substate types for the DNA metabarcoding and the LM dataset. In case of significance, an analysis of similarity percentages (SIMPER) was conducted to identify the taxa contributing most to the differences in community composition. For both the PERMANOVA and the SIMPER analyses the R package vegan was used.

Results

Morphological inventory

In total, 142 diatom taxa were identified through counts of valves in LM, 50 to genus level and 88 to species level (Table 2, Figs 2–7, 8A). The number of taxa per sample ranged between 2 and 52 with an average of 20 per sample. The additional 23 taxa were found by scanning the whole slides under LM to look for rare taxa, whereby 11 could be unambiguously assigned to a species name (Table 2, Figs 2–7, 8A). In marine samples 116 taxa were found, in the freshwater samples 93 taxa and in brackish water samples 21 taxa.

The most abundant taxa (> 2% of all counts per habitat, Table 3) across marine samples were in decreasing order *Navicula* cf. *perminuta*, *Minidiscus chilensis* P. Rivera, *Navicula* sp. 5, *Pseudogomphonema kamtschaticum* (Grunow) Medlin, *Achnanthes vicentii* Manguin, *Gyrosigma* sp., *Synedropsis* cf. *recta* and *Cocconeis fasciolata* (Ehrenberg) N.E.Brown. Across brackish water samples *Navicula gregaria* Donkin, *Navicula australoshetlandica* Van de Vijver, *Chamaepinnularia australis* Schimani & N.Abarca, *Nitzschia* cf. *gracilis*, *Nitzschia* sp. 6, *Halamphora ausloosiana* Van de Vijver & Kopalová and *Planothidium australe* (Manguin) Le Cohu were the most abundant taxa. Across the freshwater samples *Nitzschia annewillemsiana* Hamsher, Kopalová, Kociolek, Zidarova & Van de Vijver, *Nitzschia kleinteichiana* Hamsher, Kopalová, Kociolek, Zidarova & Van de Vijver, *Mayamaea sweetloveana* Zidarova, Kopalová & Van de Vijver, an unidentified centric diatom, *Nitzschia soratensis* E.A.Morales & M.L.Vis, *Psammothidium papilio* (D.E.Kellogg, Stuiver, T.B.Kellogg & G.H.Denton) K. Kopalová & Van de Vijver, *Achnanthidium* cf. *maritimo–antarcticum*, *Fragilaria* cf. *parva*, *Planothidium quadripunctatum* (D.R.Oppenheim) Sabbe, *Planothidium rostrolanceolatum* Van de Vijver, Kopalová & Zidarova and *Nitzschia* cf. *gracilis* were the most abundant taxa.

Antarctic taxonomic reference library

A total of 162 clonal cultures were established, resulting in the identification of 60 taxa: 33 of those taxa could be identified to species level, 23 to genus level and 4 where the genus affiliation is inconclusive (Table 4).

From the 60 taxa, only six had a sequence record in the International Nucleotide Sequence Database Collaboration (INSDC) databases (DDBJ, EMBL–EBI and NCBI) and 54 are new sequenced taxa. Some sequences from our Antarctic cultures were already published with a thorough morphological examination and in two cases with the description of a new species (Prelle et al. 2022; Juchem et al. 2023; Schimani et al. 2023).

Table 2. List of all taxa observed in light microscopy (LM) with author, references and morphometric information. (R) behind the taxa indicates that it was a rare species just observed in a thorough scan of the slide.

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
Achnanthes bongrainii	(M. Peragallo) A. Mann	Peragallo 1921: p. 11, pl: I figs 4–6; as *A. brevipes* in Scott and Thomas 2005: p. 121, fig. 2.65; Zidarova et al. 2022: p. 91, fig. 3	27.2–50.3	7.6–11.1		6–8	6–7		
Achnanthes vicentii	Manguin	Manguin 1957: p. 124, pl. V, fig. 26a–e; Zidarova et al. 2022: p. 93, fig. 4D–G	4.6–16.2	4.0–7.1		12–16	11–16		
Achnanthes sp. 1			21.8–32.4	8.4–10.3		8–10	8		
Achnanthes sp. 2			16.2–47.8	6.2–10.5		6–8	6–8		
Achnanthes sp. 3			31.8–34.3	4.0–5.5		11	9–10		
Achnanthes sp. 4 (R)			13.9–23.4	4.3–4.5		10	8–10		
Achnanthes sp. 5 (R)			48.5	9.8		6			
Achnanthidium australexiguum	Van de Vijver	Taylor et al. 2014: p. 47, figs 65–92	13.1–16.9	5.6–7.4		26–28	24–26		
Achnanthidium cf. *maritimo-antarcticum*	Van de Vijver & Kopalová	Van de Vijver and Kopalová 2014: p. 6, figs 29–53	14.0–16.9	2.3–2.6		28–32			
Actinocyclus actinochilus	(Ehrenberg) Simonsen	Villareal and Fryxell 1983: p. 461, figs 21–32; Scott and Thomas 2005: p. 52, fig. 2.22; Al-Handal et al. 2022: p. 85, figs 20, 21			57.5			9–10	
Amphora gourdonii	M. Peragallo	Peragallo 1921: p. 60, pl. II, fig. 23; Al-Handal and Wulff 2008b: p., fig. 85; Zidarova et al. 2022: fig. 10Y	23.2–64.4	6.6–10.8		9–13			
Amphora cf. *gourdonii* (R)	M. Peragallo	Peragallo 1921: p. 60, pl. II, fig. 23; Al-Handal and Wulff 2008b: p., fig. 85; Zidarova et al. 2022: fig. 10Y	25.7–37.3	4.8–7.2		11–16			
Amphora cf. *pusio* (R)	Cleve	Levkov 2009: 112, pl. 76, figs 22–30	21.4–30.3	3.9–6.8		13–17			
Amphora sp. (R)			37.7	7.5		11			
Australoneis frenguelliae	(Riaux-Gobin & J.M.Guerrero) J.M.Guerrero & Riaux-Gobin	Guerrero et al. 2021: figs 1–75	22.4–34.3	12.5–20.5		4–5	5–6		
Berkeleya rutilans	(Trentep. ex Roth) Grunow	Witkowski et al. 2000: p. 157, pl. 62, figs 14–17; Scott and Thomas 2005: 148, fig. 283d	21.6–24.6	5.6–6.9		28–30			
Berkeleya cf. *sparsa* (R)	Mizuno	Witkowski et al. 2000: p. 158, pl. 62, figs 7–9	24.8–35.9	5.0–6.0		22–26			
Biremis ambigua	(Cleve) D.G. Mann	Simonsen 1992: p. 42, pl. 40, figs 4–10; Witkowski et al. 2000: p. 158, pl. 155, figs 2–6; Al-Handal et al. 2022: p. 93, figs 75, 76	33.7–48.8	5.0–5.8		6–8			
Brachysira minor	(Krasske) Lange Bertalot	Lange-Bertalot and Moser 1994: p. 47, pl. 47, figs 1–8; Zidarova et al. 2016a: p. 250, pl. 110, figs 1–25	10.4–18.1	3.4–4.3					
Brandinia charcotii	(Perag.) Zidarova & P.Ivanov	Peragallo 1921: p. 68, pl. III, fig. 5; Zidarova et al. 2022, p. 94, fig. 5	68.7	8.7		13			
Caloneis australis	Zidarova, Kopalova & Van de Vijver	Zidarova et al. 2016b: p. 40, figs 1–17	25.6	4.3		22			
Chamaepinnularia australis	Schimani & N. Abarca	Schimani et al. 2023: p. 8, figs 7–9	9.7–19.2	4.2–5.5		18–24			
Chamaepinnularia gerlachei	Van de Vijver & Sterken	Van de Vijver et al. 2010: p. 432, figs 1–18	9.0–21.8	3.1–5.2		16–20			
cf. *Chamaepinnularia*			17.7–39.9	3.6–5.1		14–15			

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
cf. *Cocconeis* 1			10.6–22.2	6.5–15.2		14–19	14–18		
Cocconeis antiqua	Tempère & Brun	Romero 2011: p. 185, figs 13–35	49.3–79.0	31.1–51.5		11–15	13–19		
Cocconeis californica	Grunow	Witkowski et al. 2000: p. 102, pl. 36, figs 29, 30, pl. 42, figs 8–15; Riaux-Gobin and Romero 2003: p. 21, pl. 8–10	11.2–24.8	6.4–15.5		17–20	11–16		
Cocconeis costata	Gregory	Riaux-Gobin and Romero 2003: p. 22, pl. 1–2; Al-Handal and Wulff 2008b: p. 425, figs 43, 44; Zidarova et al. 2022: fig. 8D	14.9–30.4	8.3–15.0		10–12	8–10		
Cocconeis dallmannii	Al-Handal, Riaux-Gobin, Romero & Wulff	Al-Handal et al. 2008: p. 275, figs 33–48	11.9–20.7	8.2–14.9		13–19	10–12		
Cocconeis fasciolata	(Ehrenberg) Brown	Riaux-Gobin and Romero 2003: p. 26, pl. 19; Scott and Thomas 2005: p. 127, fig. 2.68a–d; Al-Handal and Wulff 2008b: p. 426, figs 45, 51, 52; Zidarova et al. 2022: fig. 8F, G	21.7–45.0	12.4–28.3		5–6	5–7		
Cocconeis imperatrix	A. Schmidt	Manguin 1960: p. 305, pl. 24, figs 358, 359; Riaux-Gobin and Romero 2003: p. 28, pl. 21, figs 1–8; Al-Handal and Wulff 2008b: 426, figs 46–49, 55, 56; Al-Handal et al. 2022: p. 91, fig. 52	47.2–68.8	31.8–44.4		4–5	4–5		
Cocconeis infirmata	Manguin	Manguin 1957: p. 123, pl. V, fig. 24a–c	10.7–24.9	6.0–17.4			8–16		
Cocconeis matsii	(Al-Handal, Riaux-Gobin & Wulff) Riaux-Gobin, Compère, Romero & D.M.Williams	Al-Handal et al. 2010: p. 6, figs 13–15, 25–30	9.2–19.4	5.7–11.9			5–8		
Cocconeis melchioroides	Al-Handal, Riaux-Gobin, Romero & Wulff	Al-Handal et al. 2008: p. 271, figs 2–15, 18–32	9.9–20.4	7.0–10.6		12–14	6–10		
Cocconeis pottercovei	Al-Handal, Riaux-Gobin et Wulff	Al-Handal et al. 2010: p. 3, figs 2–12, 19–24	11.2–14.8	7.1–8.9		11–13	10–12		
Corethron pennatum	(Grunow) Ostenfeld	Van Heurck 1909: p. 30, pl. VI, fig. 86; Crawford et al. 1998: p. 5, figs 1, 6–25			17.8				
Craspedostauros laevissimus	(West & G.S.West) Sabbe	Sabbe et al. 2003: p. 235, figs 35–37, 85; Van de Vijver et al. 2012: p. 154, figs 24–39	30.2–49.4	4.7–5.6		26–29			
Diploneis sp.			17.8	6.6		16			
Ellerbeckia sol	(Ehrenberg) R.M.Crawford & P.A.Sims	as *Melosira sol* in Scott and Thomas 2005: p. 66, fig. 2.32; Al-Handal et al. 2022: p. 85, figs 9, 10			94.4–102.6				
Encyonema ventricosum	(C.Agardh) Grunow	Lange-Bertalot et al. 2017: p. 209, pl. 89, figs 18–22	12.9–23.4	4.7–6.4		15–19			
Entomoneis sp.			52.1–53.2	6.5–11.1		30			
Entopyla ocellata	(Arnott) Grunow	Al-Handal and Wulff 2008b: p. 427, figs 57–62; Al-Handal et al. 2022: p. 89, figs 37, 38, 109–111	60.8	16.6			3		
Fallacia marnieri	(Manguin) Witkowski, Lange-Bertalot & Metzeltin	as *Navicula marnieri* in Manguin 1957: p. 127, pl. 5, figs 35a, b; Witkowski 2000: p. 207, pl. 71, figs 1–3; Al-Handal and Wulf 2008b: p. 427, figs 105, 106; Zidarova et al. 2022, fig. 9A	10.1(6.1)–24.5	5.4(3.8)–11.0		9–14(15)			
Fragilaria cf. *parva*	Tuji & D.M.Williams	Zidarova et al. 2016a: p. 36–40, pl. 3–5	16.1–52.1	2.6–4.8		15–20			
Fragilaria cf. *striatula*	Lyngbye	Zidarova et al. 2022: p. 96, figs AP–R	43.0–51.7	7.5–8.1		13–14			
Fragilariopsis curta	(Van Heurck) Hustedt	Hustedt 1958: p. 160, pl. 11, figs 140–144, pl. 12, fig. 159; Scott and Thomas 2005: p. 171, fig. 2.99; Cefarelli et al. 2010: p. 1466, figs 2a–d, 7a, b	11.7–31.4	5.7–6.6		10–13			

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
Fragilariopsis cylindrus	(Grunow ex Cleve) Helmcke & Krieger	Scott and Thomas 2005: p., fig. 2.100; Cefarelli et al. 2010: p. 1470, figs 2e–1, 7c–e	3.7–16.4	2.4–3.4		15–16			
Fragilariopsis kerguelensis	(O'Meara) Hustedt	Scott and Thomas 2005: p. 183, fig. 2.101; Cefarelli et al. 2010: p. 1470, figs 3a–h, 7f, g	25.8–27.5	7.8–8.7		5–6		11	
Fragilariopsis rhombica	(O'Meara) Hustedt	Scott and Thomas 2005: p. 179, fig. 2.104; Cefarelli et al. 2010: p. 1475, fig. 5a–e	12.6–33.2	8.4–11.6		11–16			
Fragilariopsis separanda	Hustedt	Hustedt 1958: p. 165, pl. 10, figs 108–112; Scott and Thomas 2005: p. 184, fig. 2.104; Cefarelli et al. 2010: p. 1476, fig. 6a–d	11.8–15.3	7.6–9.1		8–13			
cf. *Gedaniella*			9.3–18.9	2.4–4.4			14–18		
Gomphonema maritimo-antarcticum	Van de Vijver, Kopalová, Zidarova & Kociolek	Van de Vijver et al. 2016a: p. 212, figs 22–74	15.3–39.7	4.7–7.5		10–15			
Gomphonemopsis ligowskii	Al-Handal & E.W.Thomas	Al-Handal et al. 2018: p. 98, figs 2–25	11.4–16.3	2.1–2.9		14–16			
Gyrosigma cf. *fasciola*	J.W. Griffith & Henfrey	Jahn et al. 2005: p. 306, figs 1–7; Al-Handal and Wulff 2008a: fig. 101; Al-Handal et al. 2022: p. 94, fig. 80	101.2–172.8	12.4–15.9		20–22			
Gyrosigma tenuissimum var. *angustissimum*	Simonsen	Simonsen 1959: p. 83, pl 12, fig. 7; Cardinal 1986: p. 179, figs 37, 38	155.2–159.6	7.4		19			
Gyrosigma sp.			158.0–256.9	15.5–20.4		22–24			
cf. *Halamphora* (R)			21.7	3.0					
Halamphora ausloosiana	Van de Vijver & Kopalová	Van de Vijver et al. 2014a: p. 379, figs 4S–AG, 6	16.4–36.5	4.5–6.9		22–24			
Halamphora lineata	(Gregory) Levkov	Levkov 2009: p. 202, pl. 101, figs 12–19	37.0–44.0	5.8–7.1		15			
Halamphora cf. *staurophora*	(Juhlin-Dannfelt) Álvarez-Blanco & S.Blanco	Witkowski et al. 2000: p. 150, pl. 163, figs 34, 35; Álvarez and Blanco 2014: p. 65, pl. 36, figs 7, 8	13.7–21.0	3.3–3.6		24			
Halamphora cf. *veneta* (R)	(Kützing) Levkov	Levkov 2009: p. 242, pl. 94, figs 9–19, p. 102, figs 17–30	39.1	5.8		23			
Halamphora sp. 1 (R)			36.9	7.6		16			
Halamphora sp. 2			34.9–38.3	4.4–6.2		11–14			
Halamphora sp. 3 (R)			17.4–21.0	4.3–5.0		14			
Hantzschia amphioxys (R)	(Ehrenberg) Grunow	Lange-Bertalot et al. 2017: p. 338, pl. 104, figs 1–5	31.5–49.8	6.1–6.3		21–22			4–6
Hantzschia hyperaustralis	Van de Vijver & Zidarova	Zidarova et al. 2010: p. 326, fig. 6A–I	79.7–109.2	12.4–14.8		20–21			4–7
Hantzschia cf. *virgata*	(Roper) Grunow	Witkowski et al. 2000: p. 364, pl. 175, fig. 10, pl. 176, figs 1–3; Sabbe et al. 2003: p. 238, fig. 59; Silva et al. 2019: p. 800, fig. 2(22)	72.3–81.6	7.7–9.0		11–13		24	6
Hippodonta hungarica	(Grunow) Lange-Bertalot, Metzeltin & Witkowski	Zidarova et al. 2016a: p. 124, pl. 47	12.4–18.2	4.8–5.4		9–10			
Humidophila sceppacuerciae	Kopalová	Kopalová et al. 2015: p. 121, figs 2–26	7.7–9.6	2.1–3.1					
Humidophila tabellariaeformis	(Krasske) R.L. Lowe et al.	Zidarova et al. 2016a: p. 234, pl. 102	13.9–15.0	4.9–5.1		25–26			
Licmophora antarctica	M. Peragallo	Fernandes et al. 2014: p. 469, figs 1–9	47.1–100.5	9.6–12.6		6–7			
Licmophora belgicae (R)	M. Peragallo	Fernandes et al. 2014: p. 470, figs 10–20	134.6	15.6		11			
Licmophora cf. *gracilis*	(Ehrenberg) Grunow	Witkowski et al. 2000: p. 65, pl. 18, figs 12–15, pl. 19, figs 7–15; Al-Handal and Wulff 2008b: p. 429, figs 6–8; Fernandes et al. 2014: p. 471, figs 21–29; Al-Handal et al. 2022: p. 88, fig. 33	22.2–56.9	5.0–12.2		17–25			

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
Luticola australomutica	Van de Vijver	Van de Vijver and Mataloni 2008: p. 458, figs 39–51	18.8	6.7		20			
Luticola austroatlantica (R)	Van de Vijver, Kopalová, Spaulding & Esposito	Esposito et al. 2008: p. 1383, figs 9–27	21.4–23.6	7.4		16			
Luticola desmetii	Kopalová & Van de Vijver	Kopalová et al. 2011: p. 47, figs 2–13	21.9–29.3	10.6–12.6		15–16			
Luticola higleri	Van de Vijver, Van Dam & Beyens	Van de Vijver et al. 2006: p. 71, figs 3–42	10.7–28.5	7.2–10.3		12–18			
Luticola cf. *muticopsis*	(Van Heurck) D.G. Mann	Zidarova et al. 2016a: p. 188, pl. 79	13.7–20.0	6.6–8.2		16			
Luticola cf. *truncata*	Kopalová & Van de Vijver	Kopalová et al. 2009: p. 118, figs 34–50	13.7–20.0	6.6–8.2		16			
Mayamaea cf. *permitis*	(Hustedt) K.Bruder & Medlin	Lange-Bertalot et al. 2017: p. 366, pl. 50, figs 13–19; Zidarova et al. 2016a: p. 260, pl. 115, figs 1–19	6.4–7.3	3.3–3.5					
Mayamaea sweetloveana	Zidarova, Kopalová & Van de Vijver	Zidarova et al. 2016b: p. 43, figs 46–58	6.8–7.7	3.8–4.7		20–26			
Minidiscus chilensis	Rivera	Rivera and Kock 1984: p. 281, pl. 2, 3, figs 5–14; Kang et al. 2003: p. 95, figs 2, 3; Kaczmarska et al. 2009: p. 463, figs 1, 2			2.9–3.5				
Navicula australoshetlandica	Van de Vijver	Van de Vijver et al. 2011: p. 287, figs 2–15	13.0–30.5	4.5–6.0		12–15			
Navicula concordia	Riaux-Gobin & Witkowski	Witkowski et al. 2010: p. 121, figs 8–24	19.5–30.5	4.7–6.9		13–15			
Navicula cremeri	Van de Vijver & Zidarova	Van de Vijver et al. 2011: p. 289, figs 30–45	27.3	5.5		12			
Navicula criophiliforma	Witkowski, Riaux-Gobin & Daniszewska-Kowalczyk	Witkowski et al. 2010: p. 121, figs 25–38	23.3–55.9	5.8–8.5		11–13			
Navicula directa	(W.Smith) Ralfs	Witkowski et al. 2000: p. 275, pl. 129, fig. 1, pl. 133, figs 10–12; Scott and Thomas 2005: p. 157, fig. 2.87a–d; Al-Handal and Wulff 2008a: p. 64–66, 95; Zidarova et al. 2022: fig. 9N	67.7–123.6	8.2–13.1		7–9			
Navicula glaciei	Van Heurck	Van Heurck 1909: p. 11, pl. I, fig. 13; Scott and Thomas 2005: p. 158, fig. 2.89; Zidarova et al. 2022: fig. 9H	16.3–25.6	5.2–6.7		13–18			
Navicula gregaria	Donkin	Van de Vijver et al. 2002: p. 64, pl. 35, figs 9–18, pl. 36, fig. 3	16.4–25.6	5.1–6.6		16–20			
Navicula cf. *pagophila* var. *manitounukensis* (R)	Poulin & Cardinal	Poulin and Cardinal 1982: p. 2836, fig. 3; Witkowski et al. 2000: p. 293, p. 128, figs 4–6	27.0–32.1	10.8–12.8		21–26			
Navicula cf. *perminuta*	Grunow	Busse and Snoeijs 2002: p. 277, figs 11–15, 34–40; Lange-Bertalot et al. 2017: p. 400, pl. 30, figs 25–32; Al-Handal et al. 2022: p. 92, figs 69, 70	5.5–19.9	1.9–5.0		12–20			
Navicula sp. 2			20.9–48.6	3.6–6.6		12–16			
Navicula sp. 3			18.0–31.5	3.9–5.4		10–15			
Navicula sp. 4			14.2–24.0	2.8–3.7		11–14			
Navicula sp. 5			16.9–48.9	4.4–7.4		10–14			
Navicula sp. 6			21.8–24.4	3.8–4.3		12–14			
Navicula sp. 7			14.4–28.1	4.3–5.2		12–15			
Navicula sp. 8			17.8–29.6	3.0–4.7		9–11			
Navicula sp. 9			14.0–17.3	2.9–3.3		19–21			

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
Navicula sp. 10			40.3–57.7	5.8–7.4		8–9			
Navicula sp. 11			27.3–31.4	4.9–5.3		8–9			
Navicula sp. 12			31.9–42.2	6.4–7.6		8–9			
Navicula sp. 13			16.5–31.9	4.5–6.3		11–14			
Navicula sp. 14			6.0–13.5	3.2–5.5		14–20			
Nitzschia annewillemsiana	Hamsher, Kopalová, Kociolek, Zidarova & Van de Vijver	Hamsher et al. 2016: p. 81, figs 2–22; Zidarova et al. 2016: p. 422, pl. 194	10.6–23.1	2.9–4.1		24–26			10–12
Nitzschia kleinteichiana	Hamsher, Kopalová, Kociolek, Zidarova & Van de Vijver	Hamsher et al. 2016: p. 88, figs 77–97; Zidarova et al. 2016: p. 430, pl. 198	14.2–23.3	2.5–3.3		25–29			10–14
Nitzschia cf. *gracilis*	Hantzsch	Hamsher et al. 2016: p. 83, figs 37–59; Zidarova et al. 2016a: p. 426, pl. 196	29.0–54.7	2.5–4.3					14–18
Nitzschia homburgiensis	Lange-Bertalot	Hamsher et al. 2016: p. 86, figs 60–76; Zidarova et al. 2016: p. 428, pl. 197	29.1–39.2	3.9–5.1					10–16
Nitzschia cf. *hybrida*	Grunow	Witkowski et al. 2000: p. 386, pl. 191, figs 12–14; Al-Handal and Wulff 2008b: p. 429, fig. 117; Al-Handal and Wulff 2008a: fig. 122	59.8–73.5	5.1–6.9		24–25			8–12
Nitzschia medioconstricta	Hustedt	Hustedt 1958: p. 174, pl. 13, figs 165, 166; Scott and Thomas 2005: p. 191, fig. 2.108d–f	52.3–72.1	4.4–6.8		24–26			8–11
Nitzschia soratensis	Morales & Vis	Morales and Vis 2007: p. 128, figs 253–256, 277–280; Zidarova et al. 2016a: p. 434, pl. 200	6.4–17.1	2.6–3.5		28–30			8–12
Nitzschia sp. 1 (R)			17.6	3.5					13
Nitzschia sp. 2			23.9–31.6	2.9–4.4					10–12
Nitzschia sp. 4			32.8–44.3	4.1–6.8		24–29			8–12
Nitzschia sp. 5			41.4–48.1	3.6–3.7					9–11
Nitzschia sp. 6			22.3–28.2	4.4–5.5					14–17
Nitzschia sp. 7			12.2–24.3	3.1–5.0					12–17
Odontella litigiosa	(Van Heurck) Hoban	as *Biddulphia litigiosa* in Van Heurck 1909: p. 40, pl. 10, fig. 141; Scott and Thomas 2005, p. 48, fig. 2.20a–f; Al-Handal and Wulff 2008b: p. 430, figs 80–82; Al-Handal et al. 2022: p. 87, figs 24–26	23.6–52.5	17.6–60.7					
Orthoseira roeseana (R)	(Rabenhorst) Pfitzer	Zidarova et al. 2016a: p. 34, pl. 2			13.3				
Parlibellus cf. *schuetii* (R)	(Van Heurck) E.J.Cox	Van Heurck 1909: p. 13, pl. I, fig. 10	71.0	24.0		14			
Petroneis cf. *plagiostoma*	(Grunow) D.G.Mann	Witkowski et al. 2000: p. 329, pl. 102, figs 5, 6	36.6–48.8	18.1–20.8		10–12		6–12	
Petroneis sp. 1			19.0–22.0	7.7–8.1		19		12–20	
Petroneis sp. 2			21.3–26.6	10.2–11.1		16–19		12–20	
Pinnularia australoglobiceps	Zidarova, Kopalová & Van de Vijver	Zidarova et al. 2016a: p. 362, pl. 166	30.1–35.8	10.4–12.9		12–14			
Pinnularia australomicrostauron	Zidarova, Kopalová & Van de Vijver	Zidarova et al. 2016a: p. 364–368, pl. 167–169	24.7–63.0	9.7–12.7		12–14			
Pinnularia australorabenhorstii (R)	Van de Vijver	Van de Vijver 2008: p. 224, figs 7–15, 24–28	42.0	16.7		6–8			
Pinnularia borealis (R)	Ehrenberg	Zidarova et al. 2016a: p. 376, 378, pl. 173, 174	42.3	9.0		5–6			

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
Pinnularia parallelimarginata	Simonsen	Simonsen 1992: p. 41, pl. 42, figs 1–8	30.5	5.1		15			
Pinnularia cf. *quadratarea*	(A.Schmidt) Cleve	Witkowski et al. 2000: p. 335, pl. 155, figs 17–21; Al-Handal and Wulff 2008a: figs 76, 77; Al-Handal and Wulff 2008b: p. 430, fig. 116	18.0–79.8	6.1–10.5		8–12			
Pinnularia subantarctica var. *elongata* (R)	(Manguin) Van de Vijver & Le Cohu	Van de Vijver et al. 2002: p. 96, pl. 114, figs 1–11	25.9–32.2	5.5–6.0		14			
Placoneis australis	Van de Vijver & Zidarova	Zidarova et al. 2009: p. 301, figs 44–58, 62–64	21.4–23.0	6.5–7.4		14–18			
Planothidium australe	(Manguin) Le Cohu	Zidarova et al. 2016a: p. 98, pl. 34	12.3–22.3	7.4–9.6		13–17	14–17		
Planothidium quadripunctatum	(Oppenheim) Sabbe	Van de Vijver et al. 2002: p. 101, pl. 23, figs 42–49	8.4–9.8	3.9–4.5		16–18	16–17		
Planothidium rostrolanceolatum	Van de Vijver, Kopalová & Zidarova	Van de Vijver et al. 2013: p. 109, figs 61–84	15.0–27.5	5.3–7.9		13–16	13–16		
Planothidium wetzelii	Schimani, N.Abarca & R.Jahn	Juchem et al. 2023	10.9–18.8	5.6–6.7		14–18	14–18		
Planothidium sp.			13.6–19.9	5.6–8.6		10–13	10–12		
Pleurosigma sp. 1			189.4–225.5	20.7–20.8		13–15			
Pleurosigma sp. 2			153.2–187.2	20.7–24.4		21–22			
Porosira cf. *glacialis*	(Grunow) Jørgensen	Scott and Thomas 2005: p. 84, fig. 2.41; Al-Handal et al. 2022: p. 83, figs 2–4			19.5–81.0			18–22	
Psammothidium germainii	(Manguin) Sabbe	Van de Vijver et al. 2016b: p 146, figs 9–81	19.7	9.6		22			
Psammothidium germainioides (R)	Van de Vijver, Kopalová & Zidarova	Van de Vijver et al. 2016b: p 150, figs 108–138	15.7	6.8			28		
*Psammothidium incognitu*m	(Krasske) Van de Vijver	Van de Vijver et al. 2002: p. 105, pl. 29, figs 1–11, pl. 30, figs 1, 2; Zidarova et al. 2016a: p. 86, pl. 28	13.8–16.3	5.0–5.6					
Psammothidium manguinii (R)	(Hustedt) Van de Vijver	Van de Vijver et al. 2002: p. 106, pl. 29, figs 20–33, pl. 30, figs 5–8; Zidarova et al. 2016a: p. 88, pl. 29	14.3	6.6		23	22		
Psammothidium papilio	(D:E. Kellogg, M. Stuiver, T.B. Kellogg & G.H. Denton) Kopalová & Van de Vijver	Kopalova et al. 2012: p. 204, fig. 5Q–T; Zidarova et al. 2016a: p. 90, pl. 30	8.5–14.7	4.3–5.8		24–30	24–30		
Psammothidium rostrogermainii	Van de Vijver, Kopalová & Zidarova	Van de Vijver et al. 2016b: p 148, figs 82–107	16.0–19.3	8.1–8.8		16	18		
Pseudogomphonema kamtschaticum	(Grunow) Medlin	Medlin and Round 1986: p. 216, fig. 29; Scott and Thomas 2005: p. 163, figs 2.93, 2.94a, b; Al-Handal and Wulff 2008b: p. 439, figs 95–100; Zidarova et al. 2022: fig. 10A	9.9–51.6	3.2–7.5		10–16			
Pteroncola carlinii	Almandoz & Ferrario	Almandoz et al. 2014: p. 189, figs 1–15	5.0–23.4	2.5–3.3					
Rhabdonema sp.			134.3–135.2	21.3–25.6			5–6		
Rhoicosphenia michalii	Ligowski	Ligowski et al. 2014: p. 143, figs 1–69	20.5–27.9	3.7–5.9		7–8			
Sabbea cf. *adminii*	(D.Roberts & McMinn) Van de Vijver, Bishop & Kopalová	Bishop et al. 2019: p. 45, figs 1–-29	31.1–32.0	4.5–4.6					
Sellaphora jamesrossensis	(Kopalová, & Van de Vivjer) Van de Vivjer & C.E. Wetzel	as *Eolimna jamesrossensis* in Kopalová et al. 2009: p. 116, figs 15–33, Zidarova et al. 2016a: p. 246, pl. 108	11.8–14.2	5.5–6.0		20–22			

Taxa	Author	Reference	Length [µm]	Width [µm]	Diameter [µm]	Striae RV in 10 µm	Striae RLV in 10 µm	Areolae in 10 µm	Fibulae in 10 µm
Shionodiscus gracilis var. *expectus*	(VanLandingham) Alverson, Kang et Theriot	as *Thalassiosira gracilis* var. *expecta* in Johansen and Fryxell 1985: p. 170, figs 8, 60–63; Alverson et al. 2006: p. 259			9.9–13.6			14–18	
Stauroneis acidojarensis (R)	Zidarova, Kopalová & Van de Vijver	Zidarova et al. 2014: p. 197, figs 13–29	45.2	9		22			
Stauroneis latistauros	Van de Vijver & Lange Bertalot	Van de Vijver et al. 2004: p. 48, pl. 49; Zidarova et al. 2016: p. 318–322, pl. 144–146	26.4–35.1	7.4–8.4		20–24			
Stauroneis pseudomuriella (R)	Van de Vijver & Lange Bertalot	Vijver et al. 2004: p. 56, pl. 61; Zidarova et al. 2016: p. 330, pl. 150	21.4–29.9	4.8–5.0		22			
Staurosira pottiezii	Van de Vijver	Van de Vijver et al. 2014b: p. 257, figs 1–25	25.8	4.2			13		
Synedropsis cf. *recta*	Hasle, Medlin & Syvertsen	Hasle et al. 1994: p. 252, figs 27–30, 51–55, 57–60, 68–75	6.4–54.7	3.0–6.9			9–15		
Thalassionema gelida	M.Peragallo	Peragallo 1921: p .69, pl. III, fig. 10; Zidarova et al. 2022: p. 102, fig. 7	63.1–153.5	3.5–6.3			10–11		
Thalassiosira antarctica	Comber	Johansen and Fryxell 1985: p. 158, figs 15–17, 37–39			29.0–44.6			13–15	
Thalassiosira scotia	Fryxell & Hoban	Johansen and Fryxell 1985: p. 176, figs 25, 26, 40–42			21.9–29.1			8–9	
Trachyneis aspera	(Ehrenberg) Cleve	Witkowski et al. 2000: p. 355, pl. 139, fig. 14, pl. 159, figs 1–6, 9; Al-Handal and Wulff 2008b: p. 432, figs 89, 90, 101; Al-Handal et al. 2022: 93, fig. 74	94.0–188.7	17.5–31.7		7–8			
Trigonium arcticum	(Brightwell) Cleve	Scott and Thomas 2005: p. 18, fig. 2.6; Al-Handal et al. 2022: p. 87, fig. 22	123.0					3–4	
Tripterion cf. *margaritae*	(Frenguelli & Orlando ex Fernandes & Sar) Fernandes & Sar	Fernandes and Sar 2009: p. 67, figs 2–62	12.1–16.2	3.2–4.1		24–25			
Unidentified centric diatom					2.8–4.5				
Unidentified pennate diatom			12.4	3.0			12		

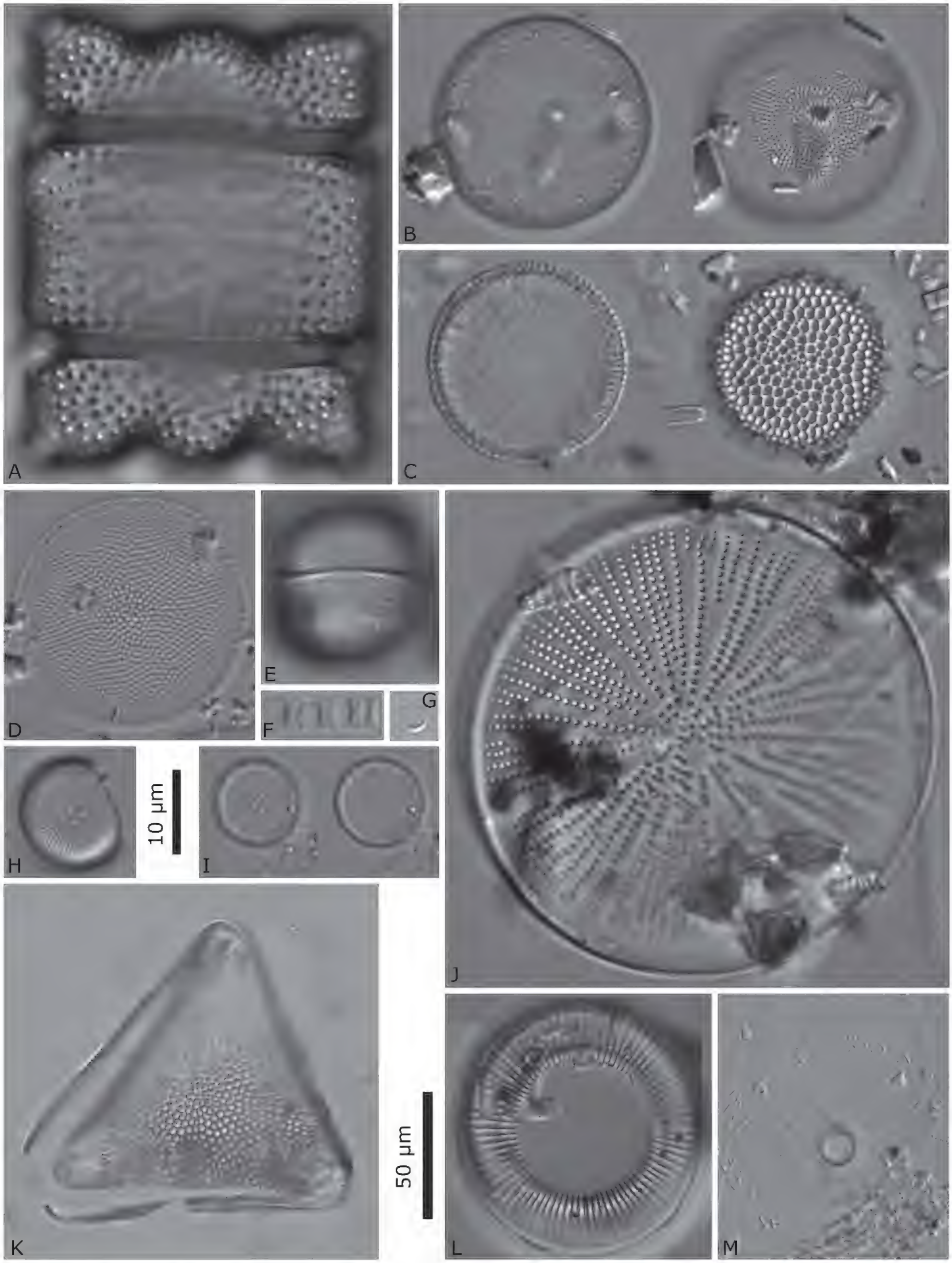

Figure 2. LM pictures of taxa found by morphological analyses. **A** *Odontella litigiosa*. **B** *Porosira* cf. *glacialis*. **C** *Thalassiosira scotia*. **D** *Thalassiosira antarctica*. **E** *Melosira* sp. **F** Unidentified centric diatom. **G** *Minidiscus chilensis*. **H** *Orthoseira roeseana*. **I** *Shionodiscus gracilis* var. *expectus*. **J** *Actinocyclus actinochilus*. **K** *Trigonium arcticum*. **L** *Ellerbeckia sol*. **M** *Corethron pennatum*. Scale bars: 10 µm (**A–J**); 50 µm (**K–M**).

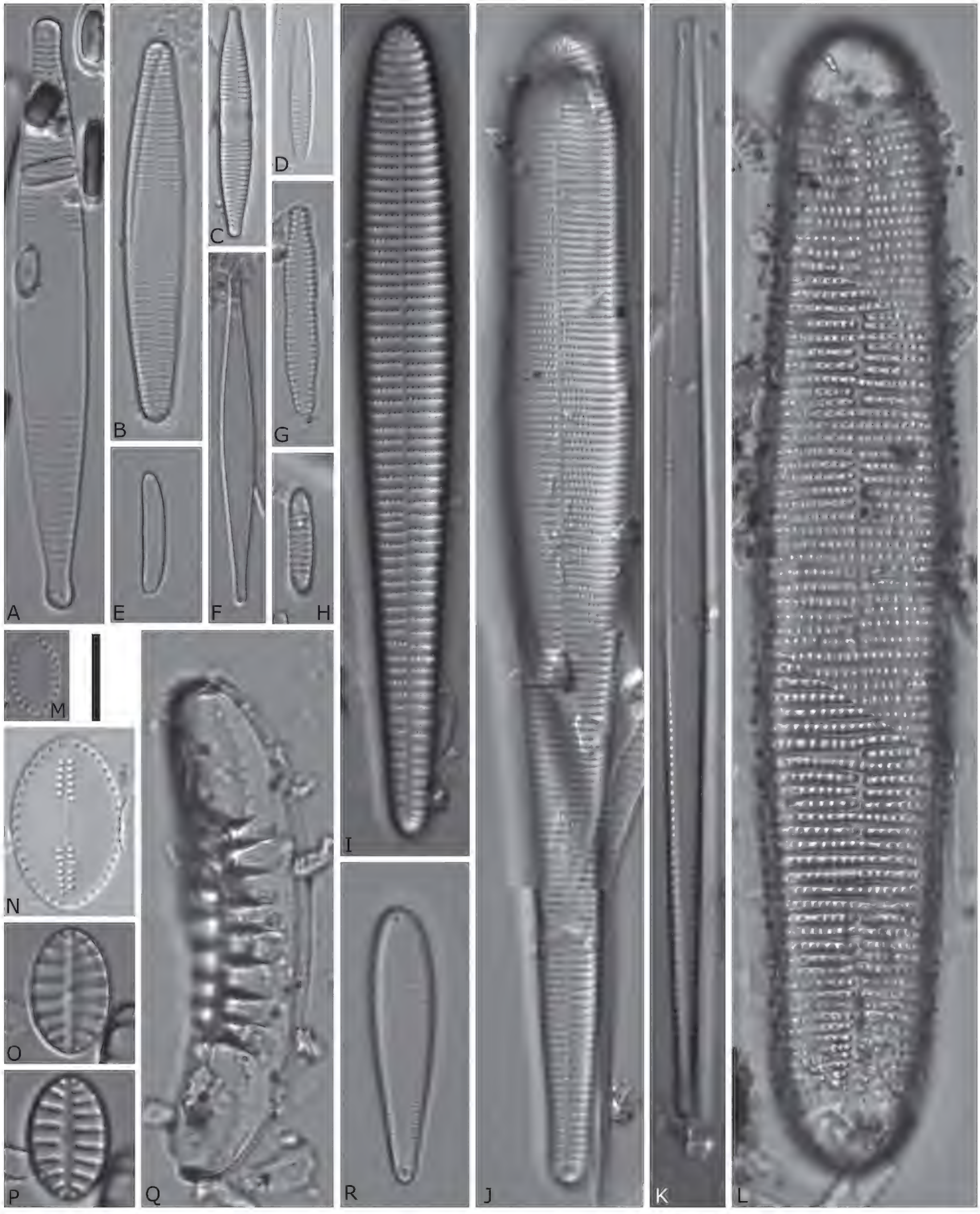

Figure 3. LM pictures of taxa found by morphological analyses. **A** *Brandinia charcotii*. **B** *Fragilaria* cf. *striatula*. **C** *Fragilaria* cf. *parva*. **D** cf. *Gedaniella*. **E** *Pteroncola carlinii*. **F** *Synedropsis* cf. *recta*. **G** *Staurosira pottiezii*. **H** Unidentified pennate diatom. **I** *Licmophora antarctica*. **J** *Licmophora belgicae*. **K** *Thalassionema gelida*. **L** *Rhabdonema* sp. **M** *Cocconeis pottercovei*. **N** *Cocconeis infirmata*. **O, P** *Cocconeis matsii*. **Q** *Entopyla ocellata*. **R** *Licmophora* cf. *gracilis*. Scale bar: 10 µm.

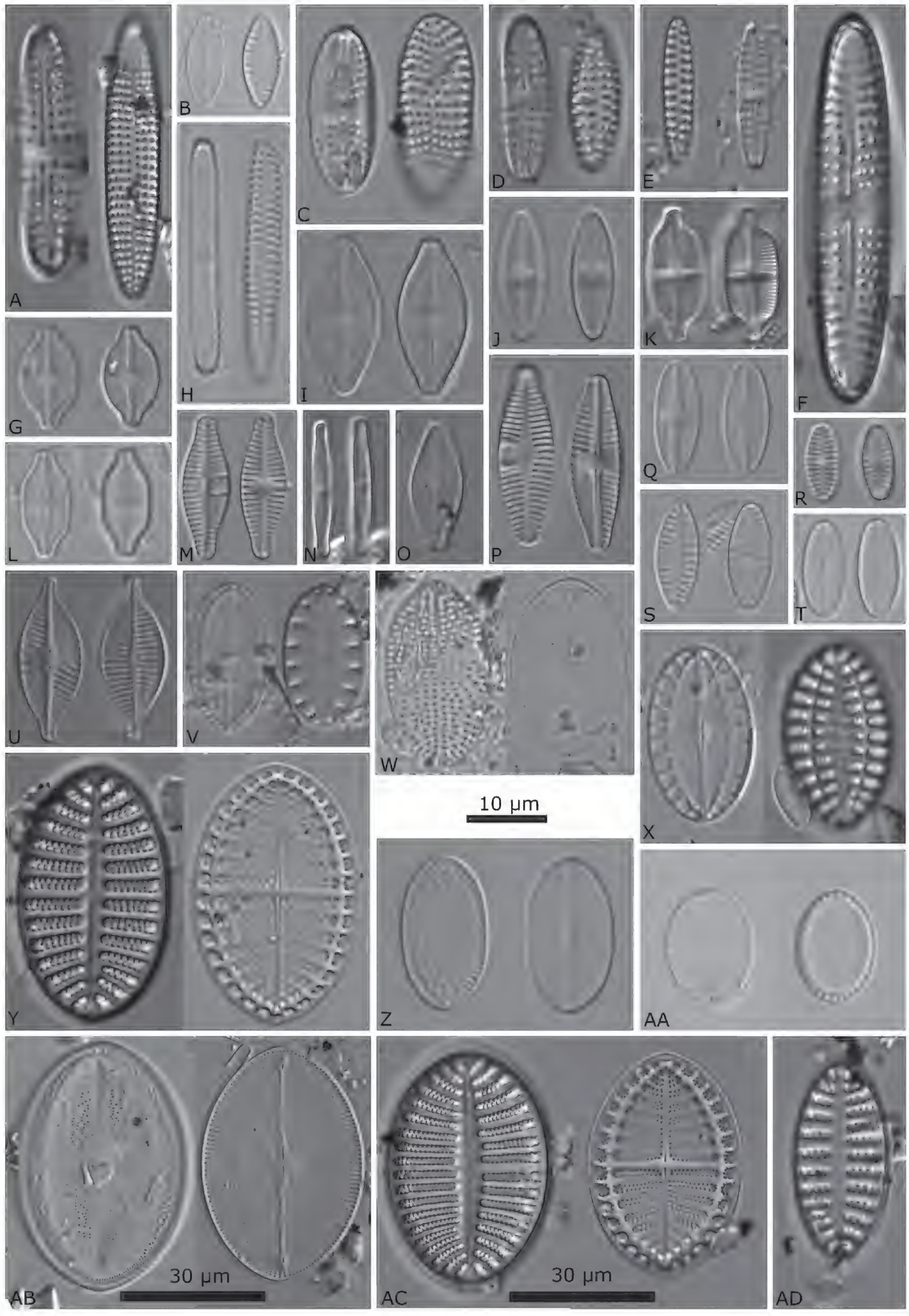

Figure 4. LM pictures of taxa found by morphological analyses. **A** *Achnanthes bongrainii*. **B** *Achnanthes vicentii*. **C** *Achnanthes* sp. 1. **D** *Achnanthes* sp. 2. **E** *Achnanthes* sp. 4. **F** *Achnanthes* sp. 5. **G** *Psammothidium rostrogermainii*. **H** *Achnanthes* sp. 3. **I** *Psammothidium germainii*. **J** *Psammothidium incognitum*. **K** *Achnanthidium australexiguum*. **L** *Psammothidium manguinii*. **M** *Planothidium wetzelii*. **N** *Achnanthidium* cf. *maritimo-antarcticum*. **O** *Psammothidium germainioides*. **P** *Planothidium rostrolanceolatum*. **Q** *Psammothidium papilio*. **R** *Planothidium quadripunctatum*. **S** *Planothidium* sp. **T** cf. *Cocconeis* 2. **U** *Planothidium australe*. **V** *Cocconeis melchioroides*. **W** *Cocconeis californica*. **X** *Australoneis frenguelliae*. **Y** *Cocconeis fasciolata*. **Z** cf. *Cocconeis* 1. **AA** *Cocconeis dallmannii*. **AB** *Cocconeis antiqua*. **AC** *Cocconeis imperatrix*. **AD** *Cocconeis costata*. Scale bars: 10 µm (**A–AA, AD**); 30 µm (**AB, AC**).

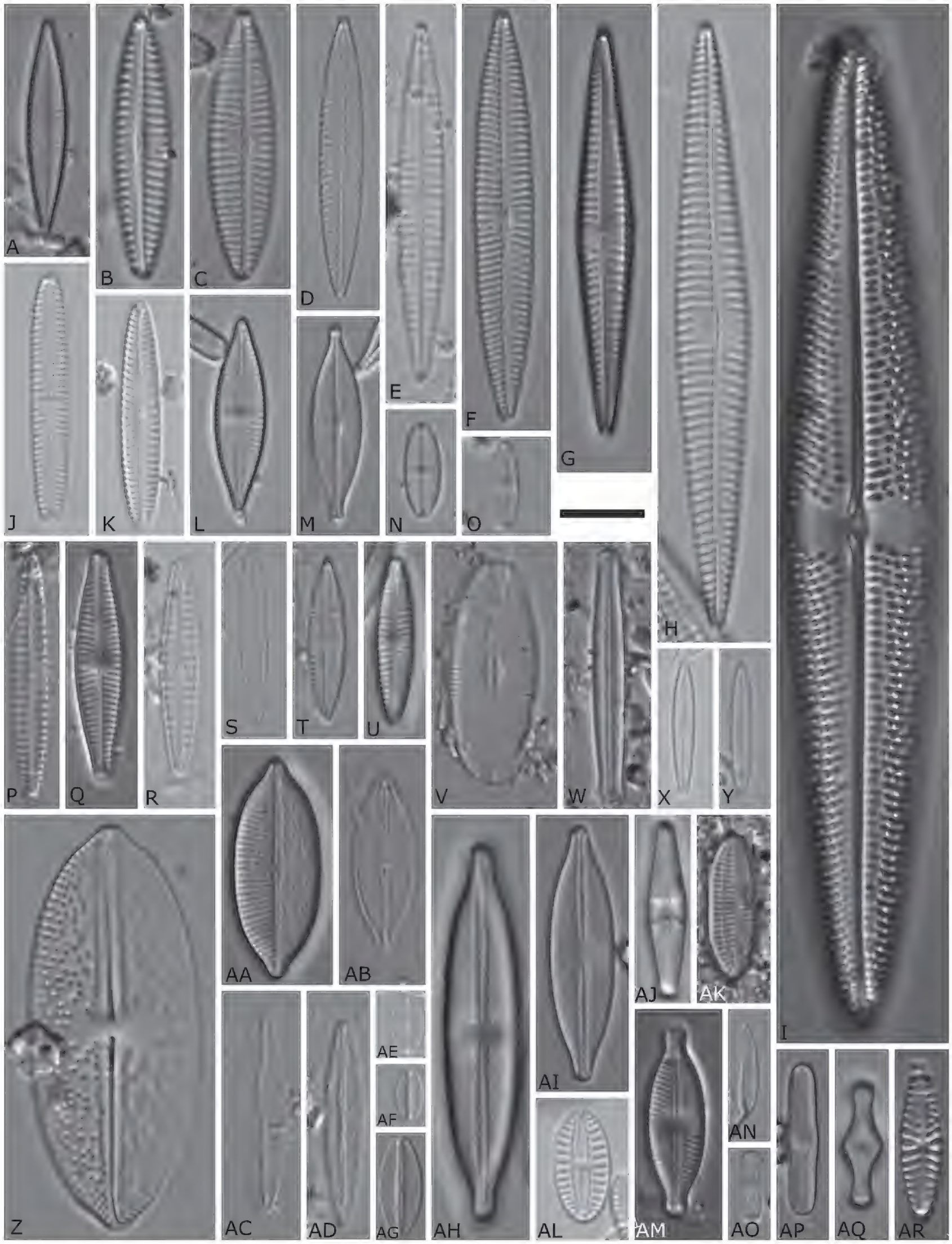

Figure 5. LM pictures of taxa found by morphological analyses. **A** *Navicula* sp. 3. **B** *Navicula* sp. 12. **C** *Navicula* sp. 1. **D** *Navicula* sp. 5. **E** *Navicula* sp. 10. **F** *Navicula criophiliforma*. **G** *Navicula* sp. 2. **H** *Navicula directa*. **I** *Trachyneis aspera*. **J** *Navicula concordia*. **K** *Navicula* sp. 13. **L** *Navicula glaciei*. **M** *Navicula gregaria*. **N** *Navicula* sp. 14. **O** *Navicula* cf. *perminuta*. **P** *Navicula* sp. 8. **Q** *Navicula cremeri*. **R** *Navicula* sp. 11. **S** *Navicula* sp. 6. **T** *Navicula* sp. 7. **U** *Navicula australoshetlandica*. **V** *Navicula* cf. *pagophila* var. *manitounukensis*. **W** *Sabbea* cf. *adminii*. **X** *Navicula* sp. 9. **Y** *Navicula* sp. 4. **Z** *Petroneis* cf. *plagiostoma*. **AA** *Petroneis* sp. 2. **AB** *Petroneis* sp. 1. **AC** *Berkeleya rutilans*. **AD** *Berkeleya* cf. *sparsa*. **AE** *Mayamaea sweetloveana*. **AF** *Mayamaea* cf. *permitis*. **AG** *Sellaphora jamesrossensis*. **AH** *Stauroneis acidojarensis*. **AI** *Stauroneis latistauros*. **AJ** *Stauroneis pseudomuriella*. **AK** *Diploneis* sp. **AL** *Fallacia marnieri*. **AM** *Placoneis australis*. **AN** *Lunella* sp. **AO** *Humidophila sceppacuerciae*. **AP** *Brachysira minor*. **AQ** *Humidophila tabellariaeformis*. **AR** *Hippodonta hungarica*. Scale bar: 10 µm.

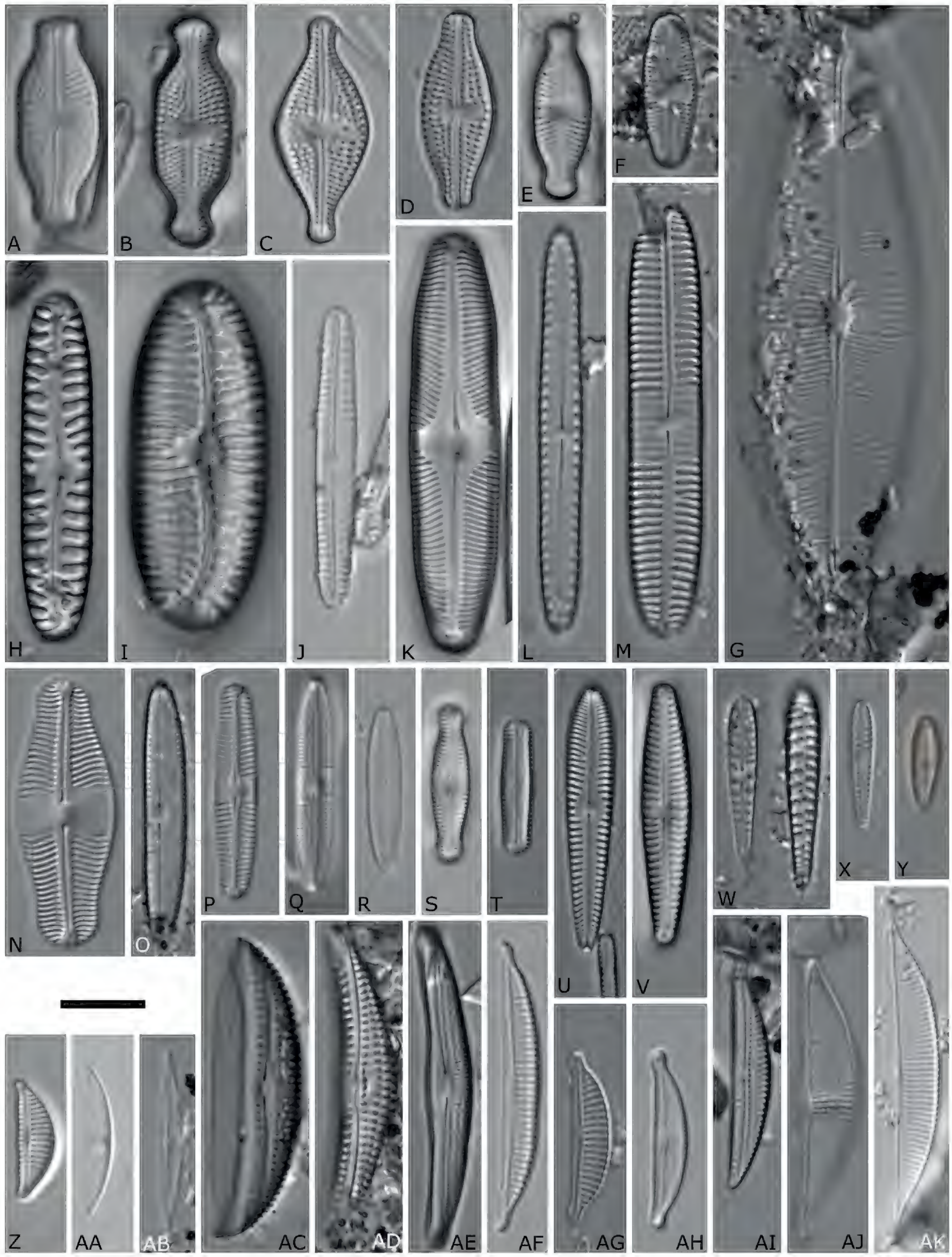

Figure 6. LM pictures of taxa found by morphological analyses. **A** *Luticola* cf. *truncata*. **B** *Luticola* cf *muticopsis*. **C** *Luticola desmetii*. **D** *Luticola higleri*. **E** *Luticola austroatlantica*. **F** *Luticola australomutica*. **G** *Parlibellus* cf. *schuetii*. **H** *Pinnularia borealis*. **I** *Pinnularia australorabenhorstii*. **J** *Pinnularia* sp. **K** *Pinnularia australomicrostauron*. **L** *Biremis ambigua*. **M** *Pinnularia* cf. *quatratarea*. **N** *Pinnularia australoglobiceps*. **O** *Pinnularia parallelimarginata*. **P** *Pinnularia subantarctica* var. *elongata*. **Q** *Caloneis australis*. **R** cf. *Chamaepinnularia*. **S** *Chamaepinnularia australis*. **T** *Chamaepinnularia gerlachei*. **U** *Pseudogomphonema kamtschaticum*. **V** *Gomphonema maritimo-antarcticum*. **W** *Rhoicosphenia michalii*. **X** *Gomphonemopsis ligowskii*. **Y** *Tripterion* cf. *margaritae*. **Z** *Encyonema ventricosum*. **AA** *Halamphora* cf. *staurophora*. **AB** cf. *Halamphora*. **AC** *Amphora gourdonii*. **AD** *Amphora* sp. **AE** *Halamphora* cf. *veneta*. **AF** *Halamphora* sp. 2. **AG** *Halamphora* sp. 3. **AH** *Halamphora ausloosiana*. **AI** *Amphora* cf. *pusio*. **AJ** *Halamphora* sp. 1. **AK** *Halamphora lineata*. Scale bar: 10 µm.

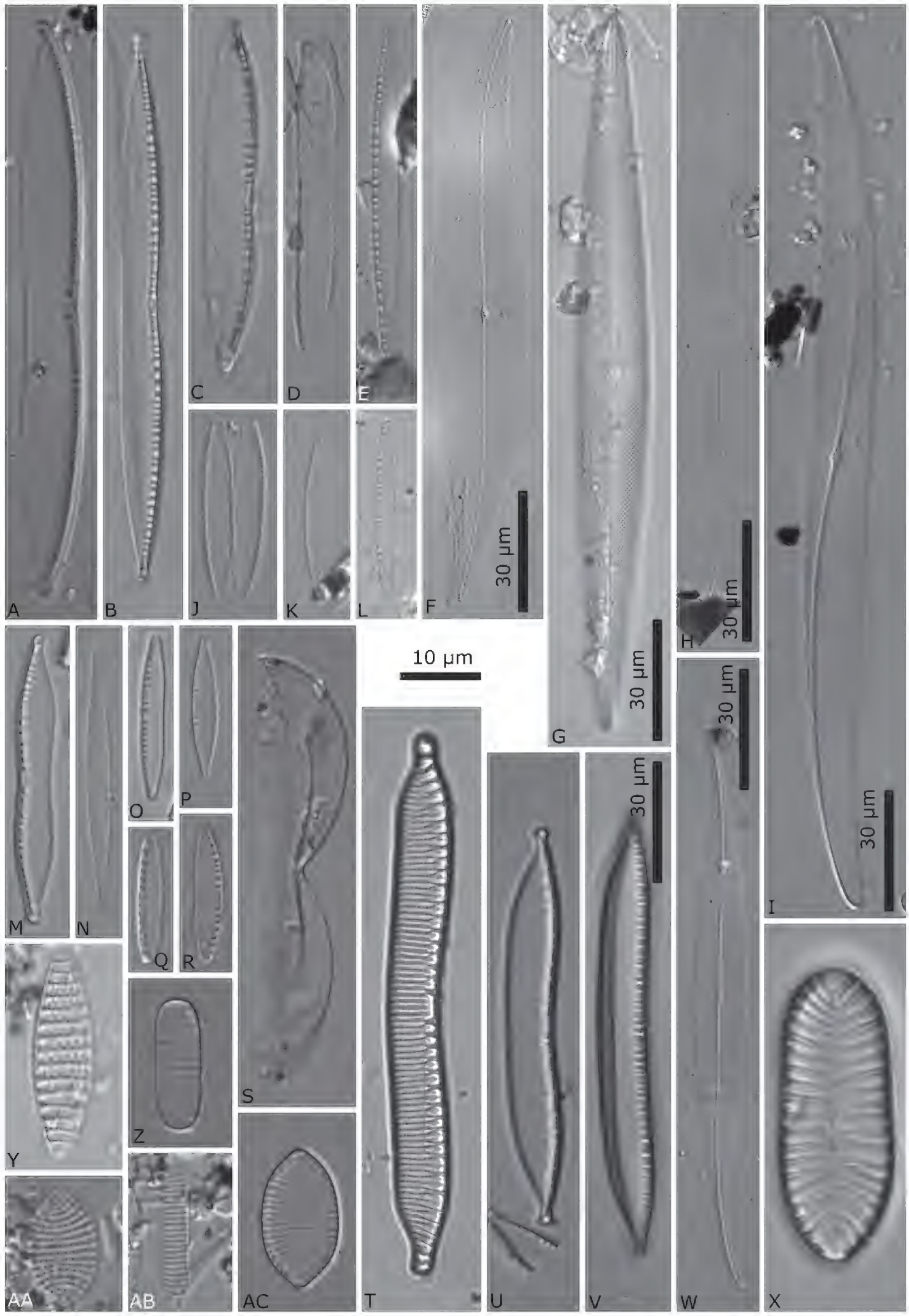

Figure 7. LM pictures of taxa found by morphological analyses. **A** *Nitzschia* cf. *hybrida*. **B** *Nitzschia medioconstricta*. **C** *Nitzschia* sp. 4. **D** *Nitzschia* sp. 3. **E** *Nitzschia* sp. 5. **F** *Pleurosigma* sp. 2. **G** *Pleurosigma* sp. 1. **H** *Gyrosigma tenuissimum* var. *angustissimum*. **I** *Gyrosigma* sp. **J** *Nitzschia* sp. 6. **K** *Nitzschia* sp. 7. **L** *Nitzschia* sp. 2. **M** *Nitzschia homburgiensis*. **N** *Nitzschia* cf. *gracilis*. **O** *Nitzschia kleinteichiana*. **P** *Nitzschia* sp. 1. **Q** *Nitzschia soratensis*. **R** *Nitzschia annewillemsiana*. **S** *Entomoneis* sp. **T** *Hantzschia* cf. *virgata*. **U** *Hantzschia amphioxys*. **V** *Hantzschia hyperaustralis*. **W** *Gyrosigma* cf. *fasciola*. **X** *Surirella australovisurgis*. **Y** *Fragilariopsis kerguelensis*. **Z** *Fragilariopsis curta*. **AA** *Fragilariopsis separanda*. **AB** *Fragilariopsis cylindrus*. **AC** *Fragilariopsis rhombica*. Scale bars: 10 µm (**A–E, J–U, X–AC**); 30 µm (**F–I, U, V**).

Table 3. Most abundant taxa (> 2% of average abundance) across marine, brackish water and freshwater samples for morphology count (LM) and metabarcoding *rbc*L and 18SV4, AA: average abundance across the habitat, NA: not taxonomically assigned. Several ASVs were assigned to the same taxon through the metabarcoding pipeline.

LM	AA [%]	*rbc*L	AA [%]	18SV4	AA [%]
Marine samples					
Navicula cf. *perminuta*	51.8	*Navicula* cf. *perminuta*	13.3	NA	24.8
Minidiscus chilensis	6.2	*Navicula* cf. *perminuta*	11.3	*Navicula* cf. *perminuta*	20.3
Navicula sp. 5	5.5	NA	11.0	NA	4.7
Pseudogomphonema kamtschaticum	4.5	NA	6.3	*Paralia sol* (syn. *Ellerbeckia sol*)	4.6
Achnanthes vicentii	3.1	NA	4.5	NA	4.4
Gyrosigma sp.	2.8	NA	3.7	*Thalassiosira minima*	2.5
Synedropsis cf. *recta*	2.2	*Navicula* cf. *perminuta*	3.2	*Navicula directa*	2.4
Cocconeis fasciolata	2.2	*Minidiscus chilensis*	2.9		
		Navicula sp.	2.9		
		Licmophora cf. *gracilis*	2.8		
		NA	2.5		
		Ellerbeckia sp.	2.2		
		NA	2.2		
Brackish water samples					
Navicula gregaria	52.3	*Navicula gregaria*	33.9	*Pinnularia australomicrostauron*	47.4
Navicula australoshetlandica	13.3	*Navicula australoshetlandica*	11.7	*Navicula gregaria*	20.6
Chamaepinnularia australis	7.1	*Nitzschia* sp.	8.8	*Navicula* cf. *veneta*	7.0
Nitzschia cf. *gracilis*	6.2	*Pinnularia australomicrostauron*	6.7	*Nitzschia* sp.	4.1
Nitzschia sp. 6	6.0	*Navicula gregaria*	6.4	*Pinnularia australomicrostauron*	2.8
Halamphora ausloosiana	5.3	*Chamaepinnularia australis*	5.2	*Pinnularia australomicrostauron*	2.6
Planothidium australe	2.2	NA	5.1		
		Nitzschia cf. *gracilis*	5.0		
		Halamphora ausloosiana	3.2		
		Pinnularia australoglobiceps	3.0		
		Nitzschia sp.	2.3		
Freshwater samples					
Nitzschia annewillemsiana	19.4	*Mayamaea sweetloveana*	13.6	*Pinnularia australomicrostauron*	28.1
Nitzschia kleinteichiana	16.0	*Fragilaria* sp.	9.9	*Nitzschia* cf. *frustulum*	10.9
Mayamaea sweetloveana	11.4	*Nitzschia* cf. *frustulum*	8.5	*Gomphonema maritimo-antarcticum*	7.7
Unidentified centric diatom	10.8	*Nitzschia kleinteichiana*	8.3	NA	6.5
Nitzschia soratensis	10.5	*Nitzschia* sp.	7.6	*Fragilaria* sp.	5.8
Psammothidium papilio	7.0	NA	6.6	*Encyonema* sp.	3.4
Achnanthidium cf. *maritimo-antarcticum*	6.1	*Nitzschia* cf. *gracilis*	6.1	*Planothidium rostrolanceolatum*	3.4
Fragilaria cf. *parva*	4.6	*Encyonema* sp.	4.9	*Nitzschia* cf. *gracilis*	2.9
Planothidium quadripunctatum	2.4	*Achnanthidium* sp.	4.2	*Achnanthidium* sp.	2.4
Planothidium rostrolanceolatum	2.1	*Mayamaea* cf. *permitis*	3.6	NA	2.3
Nitzschia cf. *gracilis*	2.0	*Gomphonema maritimo-antarcticum*	4.2	*Nitzschia* sp.	2.2
		Planothidium rostrolanceolatum	3.4	*Planothidium rostrolanceolatum*	2.2
		Planothidium cf. *pumilum*	2.6		
		Nitzschia sp.	2.0		

Table 4. Taxa which were established as clonal cultures, strain numbers, in case of publication: reference and accession number.

Taxon	Strain	Voucher at BGBM	DNA Bank	Publication of strain	Accession number *rbc*L	Accession number 18SV4
Achnanthes vicentii	D305_008	B 40 0045332	DB43189			
	D322_002	B 40 0045222	DB43092			
	D326_020	B 40 0045334	DB43015			
Brachysira minor	D300_027	B 40 0045258	DB42968			
	D300_029	B 40 0045305	DB43129			
Chaetocerus cf. *neogracilis*	D305_007	B 40 0046208	DB43188			
Chamaepinnularia australis	D294_001	B 40 0045203	DB43033	(Schimani et al. 2023)	OX386460	OX386235
	D294_002	B 40 0045204	DB43034	(Schimani et al. 2023)	OX386461	OX386236
	D294_013	B 40 0045208	DB43043	(Schimani et al. 2023)	OX386464	OX386239
	D294_014	B 40 0045209	DB43074	(Schimani et al. 2023)	OX386465	OX386240
Chamaepinnularia gerlachei	D294_005	B 40 0045272	DB43037	(Schimani et al. 2023)	OX386462	OX386237
	D294_006	B 40 0045207	DB43038	(Schimani et al. 2023)	OX386463	OX386238
	D296_001	B 40 0045355	DB43045	(Prelle et al. 2022; Schimani et al. 2023)	OX258987	OX258985
	D296_002	B 40 0045356	DB43046	(Schimani et al. 2023)	OX386466	OX386241
	D297_003	B 40 0045277	DB43047	(Schimani et al. 2023)	OX386467	OX386242
cf. *Chamaepinnularia*	D301_002	B 40 0045342	DB42990			
Cocconeis fasciolata	D326_023	B 40 0045353	DB43018			
cf. *Cocconeis* 1	D301_001	B 40 0045179	DB42989			
	D301_009	B 40 0045315	DB42997			
cf. *Cocconeis* 2	D326_035	B 40 0045271	DB43025			
	D326_037	B 40 0045328	DB43027			
	D326_038	B 40 0045350	DB43028			
	D326_039	B 40 0045329	DB43029			
Cylindrotheca cf. *closterium*	D322_018	B 40 0046211	DB43648			Not available
Fallacia marnieri	D301_003	B 40 0045314	DB42991	This study	OR355374	Not available
	D301_004	B 40 0045217	DB42992	This study	OR355375	OR352010
	D323_016	B 40 0045268	DB43144	This study	Not available	OR352011
	D326_002	B 40 0045167	DB43001	This study	OR355376	OR352012
	D326_005	B 40 0045169	DB43003	This study	OR355377	OR352013
	D326_007	B 40 0045199	DB43005	This study	OR355378	OR352014
	D326_014	B 40 0045235	DB43010	This study	OR355379	OR352015
	D326_016	B 40 0045236	DB43012	This study	OR355380	OR352016
	D326_017	B 40 0045346	DB43013	This study	OR355381	OR352017
	D326_041	B 40 0045367	DB43209	This study	OR355382	OR352018
Fragilaria cf. *parva*	D299_016	B 40 0045214	DB43076			
	D299_020	B 40 0045279	DB43080			
	D299_026	B 40 0045255	DB43087			
	D300_016	B 40 0045284	DB42962			
cf. *Gedaniella*	D291_001	B 40 0045201	DB43030			
	D293_001	B 40 0045170	DB43183			
	D324_004	B 40 0045231	DB43205			
Gomphonema maritimo-antarcticum	D299_018	B 40 0045245	DB43078	This study	OR355383	OR352019
	D299_021	B 40 0045290	DB43081	This study	OR355384	OR352020
	D299_028	B 40 0045294	DB43089	This study	Not available	OR352021
	D300_013	B 40 0045282	DB42959	This study	OR355385	OR352022
	D300_014	B 40 0045283	DB42960	This study	OR355386	OR352023
	D314_002	B 40 0045188	DB42971	This study	OR355387	OR352024
	D314_004	B 40 0045190	DB42973	This study	OR355388	OR352025
	D314_014	B 40 0045264	DB42983	This study	OR355389	OR352026
	D314_019	B 40 0045307	DB42988	This study	OR355390	OR352027

Taxon	Strain	Voucher at BGBM	DNA Bank	Publication of strain	Accession number *rbc*L	Accession number 18SV4
Halamphora ausloosiana	D294_007	B 40 0045273	DB43039	This study	OR355391	OR352028
	D294_008	B 40 0045274	DB43040	This study	OR355392	OR352029
Hantzschia hyperaustralis	D314_011	B 40 0045306	DB42980	This study	OR355393	OR352030
Humidophila sceppacuerciae	D300_002	B 40 0045280	DB42950	This study	OR355394	OR352031
	D300_022	B 40 0045302	DB42965	This study	OR355395	OR352032
Licmophora cf. *gracilis*	D308_002	B 40 0045343	DB43191			
	D308_003	B 40 0045220	DB43192			
	D308_004	B 40 0045316	DB43193			
Lunella sp.	D292_010	B 40 0045571	DB43435			
	D309_004	B 40 0045580	DB43438			
	D323_012	B 40 0045228	DB43140			
	D326_015	B 40 0045200	DB43011			
Luticola higleri	D299_001	B 40 0045311	DB43062	This study	OR355396	OR352033
	D299_010	B 40 0045312	DB43071	This study	OR355397	OR352034
Luticola desmetii	D300_028	B 40 0045313	DB43128	This study	OR355398	OR352035
Mayamaea sweetloveana	D299_006	B 40 0045175	DB43067	This study	OR355399	OR352036
	D299_007	B 40 0045176	DB43068	This study	OR355400	OR352037
	D299_009	B 40 0045178	DB43070	This study	OR355401	OR352038
	D304_001	B 40 0045246	DB42998	This study	OR355402	OR352039
	D304_002	B 40 0045259	DB42999	This study	OR355403	Not available
Mayamaea cf. *permitis*	D300_006	B 40 0045241	DB42969			
	D300_011	B 40 0045256	DB42958			
Melosira sp.	D323_018	B 40 0045309	DB43146	(Juchem et al. 2023)	OR036645	OR042180
	D323_019	B 40 0045310	DB43147			
Minidiscus chilensis	D323_014	B 40 0045229	DB43142	This study	OR355404	OR352040
	D326_021	B 40 0045325	DB43017	This study	OR355405	OR352041
Navicula australoshetlandica	D295_001	B 40 0045460	DB43327	This study	OR355406	Not available
	D300_018	B 40 0045330	DB43123	This study	OR355407	OR352042
Navicula concordia	D310_004	B 40 0045317	DB43201	(Prelle et al. 2022)	OX258991	OX259170
	D310_002	B 40 0045186	DB43199	This study	OR355408	OR352043
	D310_003	B 40 0045187	DB43200	This study	OR355409	OR352044
	D310_006	B 40 0045576	DB43439	This study	OR355410	OR352045
Navicula criophiliforma	D288_003	B 40 0045335	DB43182	(Prelle et al. 2022)	OX258986	OX259166
	D288_002	B 40 0045247	DB43181	This study	OR355411	OR352046
	D326_027	B 40 0045237	DB43021	This study	OR355412	OR352047
	D322_014	B 40 0045380	DB43102	This study	OR355413	OR352048
Navicula directa	D326_001	B 40 0045166	DB43000	This study	OR355414	OR352049
Navicula gregaria	D294_003	B 40 0045205	DB43035	This study	OR355415	OR352050
	D300_003	B 40 0045281	DB42951	This study	OR355416	OR352051
	D300_004	B 40 0045296	DB42952	This study	OR355417	OR352052
	D300_007	B 40 0045216	DB42954	This study	OR355418	OR352053
Navicula cf. *perminuta*	D323_004	B 40 0045159	DB43133			
	D323_011	B 40 0045322	DB43139			
	D326_010	B 40 0045233	DB43008			
	D326_012	B 40 0045234	DB43009			
Navicula sp. 1	D326_009	B 40 0045232	DB43007			
Navicula sp. 4	D307_001	B 40 0045475	DB43346			Not available
	D310_007	B 40 0045583	DB43440			Not available
Navicula sp. 5	D301_007	B 40 0045242	DB42969			
	D301_008	B 40 0045331	DB42996			
Navicula sp. 6	D291_006	B 40 0045474	DB43320			

Taxon	Strain	Voucher at BGBM	DNA Bank	Publication of strain	Accession number *rbc*L	Accession number 18SV4
Navicula sp. 13	D310_001	B 40 0045185	DB43198			
	D326_006	B 40 0045198	DB43004			
	D326_019	B 40 0045347	DB43014			
Nitzschia annewillemsiana	D300_012	B 40 0045357	DB43122	(Prelle et al. 2022)	OX258988	OX259167
Nitzschia cf. *gracilis*	D299_014	B 40 0045212	DB43074			
Nitzschia homburgiensis	D299_002	B 40 0045172	DB43063	This study	OR355419	OR352054
Nitzschia kleinteichiana	D314_005	B 40 0045191	DB42974	This study	OR355420	OR352055
	D314_008	B 40 0045194	DB42977	This study	OR355421	OR352056
Nitzschia medioconstricta	D309_001	B 40 0045569	DB43526	This study	OR355422	Not available
	D309_002	B 40 0045577	DB43527	This study	OR355423	Not available
Nitzschia soratensis	D300_026	B 40 0045257	DB42967	This study	OR355424	OR352057
Nitzschia sp. 3	D322_015	B 40 0045364	DB43103			
	D322_016	B 40 0045365	DB43104			
Nitzschia sp. 4	D310_008	B 40 0045584	DB43441			Not available
Nitzschia sp. 7	D324_002	B 40 0045165	DB43203			
Odontella litigiosa	D305_005	B 40 0045181	DB43186			
	D323_008	B 40 0045163	DB43137			
Pinnularia australoglobiceps	D294_004	B 40 0045206	DB43036			
Pinnularia australomicrostauron	D299_005	B 40 0045211	DB43066			
	D314_001	B 40 0045261	DB42970			
	D314_003	B 40 0045189	DB42972			
	D314_010	B 40 0045195	DB42979			
	D314_013	B 40 0045263	DB42982			
	D314_017	B 40 0045287	DB42986			
Pinnularia cf. *quadratarea*	D324_001	B 40 0045164	DB43202			
Pinnularia sp.	D322_010	B 40 0045321	DB43098			Not available
Planothidium australe	D294_010	B 40 0045275	DB43041	This study	OR355425	OR352058
	D294_011	B 40 0045276	DB43042	This study	OR355426	OR352059
	D300_005	B 40 0045297	DB42953	This study	OR355427	OR352060
Planothidium rostrolanceolatum	D299_003	B 40 0045173	DB43064	This study	OR355428	OR352061
	D299_008	B 40 0045177	DB43069	This study	OR355429	OR352062
	D299_022	B 40 0045252	DB43082	This study	OR355430	OR352063
	D300_021	B 40 0045286	DB42964	This study	OR355431	OR352064
	D314_007	B 40 0045193	DB42976	This study	OR355432	OR352065
Planothidium wetzelii	D300_015	B 40 0045340	DB42961	(Prelle et al. 2022)	OX258989	OX259168
	D300_019	B 40 0045358	DB43124	(Juchem et al. 2023)	OR036648	OR042183
	D300_020	B 40 0045301	DB43125	(Juchem et al. 2023)	OR036647	OR042182
	D300_025	B 40 0045341	DB42966	(Juchem et al. 2023)	OR036646	OR042181
Planothidium sp.	D326_029	B 40 0045349	DB43022			Not available
Pleurosigma sp. 2	D293_002	B 40 0045202	DB43184			
	D322_007	B 40 0045320	DB43097			
	D323_001	B 40 0045226	DB43130			
	D323_002	B 40 0045267	DB43131			
	D323_003	B 40 0045227	DB43132			
	D324_003	B 40 0045230	DB43204			
	D326_003	B 40 0045197	DB43002			
Porosira cf. *glacialis*	D308_005	B 40 0045182	DB43194			
	D323_005	B 40 0045160	DB43134			
Psammothidium papilio	D300_023	B 40 0045303	DB43126	(Prelle et al. 2022)	OX258990	OX259169
	D299_012	B 40 0045238	DB43072	This study	OR355433	OR352066
	D299_013	B 40 0045239	DB43073	This study	OR355434	OR352067

Taxon	Strain	Voucher at BGBM	DNA Bank	Publication of strain	Accession number *rbc*L	Accession number 18SV4
Psammothidium papilio	D299_023	B 40 0045291	DB43083	This study	OR355435	OR352068
	D299_024	B 40 0045253	DB43084	This study	OR355436	OR352069
	D299_025	B 40 0045254	DB43086	This study	OR355437	OR352070
	D300_001	B 40 0045295	DB43121	This study	OR355438	OR352071
	D300_010	B 40 0045300	DB42957	This study	OR355439	OR352072
	D314_015	B 40 0045319	DB42984	This study	OR355440	OR352073
Stauroneis latistauros	D314_009	B 40 0045318	DB42978	This study	OR355441	OR352074
	D314_016	B 40 0045344	DB42985	This study	OR355442	OR352075
Surirella australovisurgis	D300_017	B 40 0045285	DB42963			
Synedropsis cf. *recta*	D305_003	B 40 0045180	DB43185			

Sequences of taxa, where identification was possible, were submitted to GenBank. The other sequences will be published when a thorough morphological description of the species has been performed. Those sequences can be retrieved from the DNA Databank of the Botanic Garden Berlin after personal communication.

Metabarcoding Inventory

The Illumina MiSeq sequencing run generated 7,460,203 reads for the *rbc*L marker and 5,623,490 reads for the 18S V4 marker. After processing the reads through the DADA2 pipeline and improvement of the dataset by metbaR for *rbc*L 7,381,429 reads remained belonging to 1,041 ASVs and for 18S V4 5,570,517 reads remained belonging to 2,251 ASVs.

For the *rbc*L marker 6,002,917 of reads and 810 of ASVs belong to diatoms corresponding to 81.3% and 77.8% respectively. The majority of the non–diatom reads were assigned to green and brown algae. The average number of diatom–ASVs per sample ranged between 24 and 135. Of all ASVs, 283 could be assigned to a species in the reference library, whereby several ASVs were assigned to the same species and additional 156 ASVs could be assigned to genus level. In the marine samples, 611 ASVs were found; 292 ASVs could be assigned to genus level (47.8%) and 190 to species level (31.1%). In the freshwater samples, 216 ASVs were recovered; 152 could be assigned to genus level (70.4%) and 96 to species level (44.4%). Finally in the brackish water samples 52 ASVs were found; 38 could be assigned to genus level (73.0%) and 25 to species level (48.1%).

The most abundant taxa (sequence relative abundance ≥ 2%, Table 3) in decreasing order across all marine samples belong to *Navicula* cf. *perminuta*, *Minidiscus chilensis*, *Navicula* sp., *Licmophora* cf. *gracilis*, *Ellerbeckia* sp. and six taxa where no genus could be assigned. Across the brackish samples *N. gregaria*, *N. australoshetlandica*, *Nitzschia* sp., *Pinnularia australomicrostauron* Zidarova, Kopalová & Van de Vijver, *C. australis*, *Nitzschia* cf. *gracilis*, *H. ausloosiana*, *Pinnularia australoglobiceps* Zidarova, Kopalová & Van de Vijver, *Nitzschia* sp. and one unassigned taxon were the most abundant taxa. Across the freshwater samples *Mayamaea sweetloveana*, *Fragilaria* sp., *Nitzschia* cf. *frustulum*, *N. kleinteichiana*, *Nitzschia* sp., *Nitzschia* cf. *gracilis*, *Encyonema* sp., *Achnanthidium* sp., *Mayamaea* cf. *permitis*, *Gomphonema maritimo–antarcticum* Van de Vijver, Kopalová, Zidarova & Kociolek, *P. rostrolanceolatum*, *Planothidium* cf. *pumilum*, *Nitzschia* sp. and one unassigned taxon were the most abundant taxa.

For the 18S V4 marker 2,835,064 of reads and 1,439 of ASVs belong to diatoms corresponding to 50.8% and 63.9% respectively. Here as well, the majority of the non–diatom reads were assigned to green and brown algae. The average number of diatom–ASVs per sample ranged between 5 and 248. Of all ASVs 344 could be assigned to a species in the reference library, whereby several ASVs were assigned to the same species and additional 348 could be assigned to genus level. In the marine samples, 1090 ASVs were found; 462 ASVs could be assigned to genus level (42.4%) and 207 to species level (19.0%). In the freshwater samples, 300 ASVs were recovered; 211 could be assigned to genus level (70.3%) and 131 to species level (43.3%). Finally, in the brackish water samples 107 ASVs were found; 60 could be assigned to genus level (56.1%) and 36 to species level (33.6%).

The most abundant taxa (sequence relative abundance ≥ 2%, Table 3) in decreasing order across all marine samples belong to *Navicula* cf. *perminuta*, *Paralia sol* (Ehrenberg) R.M.Crawford (regarded as a synonym of *Ellerbeckia sol* (Ehrenberg) R.M.Crawford & P.A.Sims), *Thalassiosira minima* Gaarder, *Navicula directa* (W.Smith) Brébisson and 3 taxa where no genus could be assigned. Across the brackish samples *Pinnularia australomicrostauron*, *N. gregaria*, *Navicula* cf. *veneta* and *Nitzschia* sp. were the most abundant taxa. Across the freshwater samples *P. australomicrostauron*, *Nitzschia* cf. *frustulum*, *G. maritimo–antarcticum*, *Fragilaria* sp., *Encyonema* sp., *Planothidium rostrolanceolatum*, *Nitzschia* cf. *gracilis*, *Achnanthidium* sp. and *Nitzschia* sp. were the most abundant taxa.

Comparison of diatom composition of taxa from cultures, morphological and metabarcoding inventories

In the clonal cultures 60 taxa could be identified, but 51 of them were also found in the microscopy examinations of environmental samples, which means that 9 taxa were only retrieved through culturing (*Lunella* sp., cf. *Cocconeis* 2, *Chaetocerus* cf. *neogracilis*, *Cylindrotheca* cf. *closterium*, *Melosira* sp., *Navicula* sp.1, *Nitzschia* sp.3, *Pinnularia* sp., *Surirella australovisurgis* Van de Vijver, Cocquyt, Kopalová & Zidarova, Fig. 8A). The morphological analysis found 174 taxa, in contrast to the 810 and 1439 ASVs, which were recovered with *rbc*L and 18SV4 metabarcoding respectively. However, several ASVs were assigned to the same taxon from the taxonomic reference library. Therefore, 58 and 57 genera were found based on *rbc*L and 18SV4 metabarcoding respectively and 58 genera were detected by morphological identification. In total, 34 genera were retrieved in all datasets, 11 genera only by morphological identification and as well 23 only by metabarcoding (Fig. 8B). On species level 92 and 82 taxa could be assigned based on *rbc*L and 18SV4 metabarcoding respectively. The combination of the total morphological richness of 165 taxa with 73 taxa solely assigned by metabarcoding resulted in a total of 238 infrageneric taxa (Fig. 8C). Of those taxa 33 were retrieved by all three methods and 111 only by morphology. The barcode reference library of Antarctic species presented here allowed the assignment of 47 infrageneric taxa in the metabarcoding analysis, which would have been left unassigned because no matching reference sequences were available in the INSDC databases or Diat.barcode library before our study.

The relative abundances on genus level shows that in general the same genera per samples are retrieved between the three datasets (Fig. 9). However, in both the 18SV4 and the *rbc*L dataset many sequences could not be assigned to

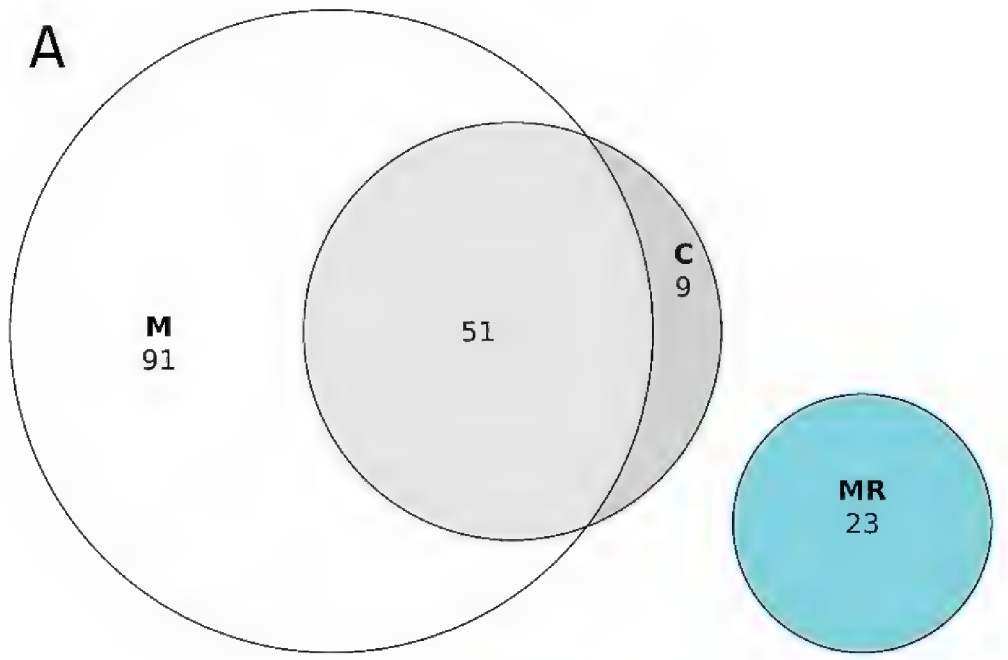

Figure 8. Venn diagrams comparing the performance of morphology and DNA metabarcoding in diatom identifications. **A** Morphological richness across all environmental samples and clonal cultures, M: infrageneric taxa identified by counting 400 valves per sample under light microscopy (LM), C: infrageneric taxa identified from clonal cultures, MR: infrageneric taxa identified by scanning LM slide for rare species. **B** Genera identified by morphology (Mor) and metabarcoding with the *rbc*L and 18SV4 marker gene. **C** Infrageneric taxa identified by morphology including rare taxa (Mor) and metabarcoding with the *rbc*L and 18SV4 marker gene (only assigned taxa to species level from metabarcoding shown).

genus level. This was especially true for the marine samples. A comparison to the morphological inventory indicates that *Gyrosigma* was underrepresented by both markers (D296, D297 and D305). In *rbc*L no reads and in 18SV4 13 reads were assigned to this genus. In some samples with a high abundance of not

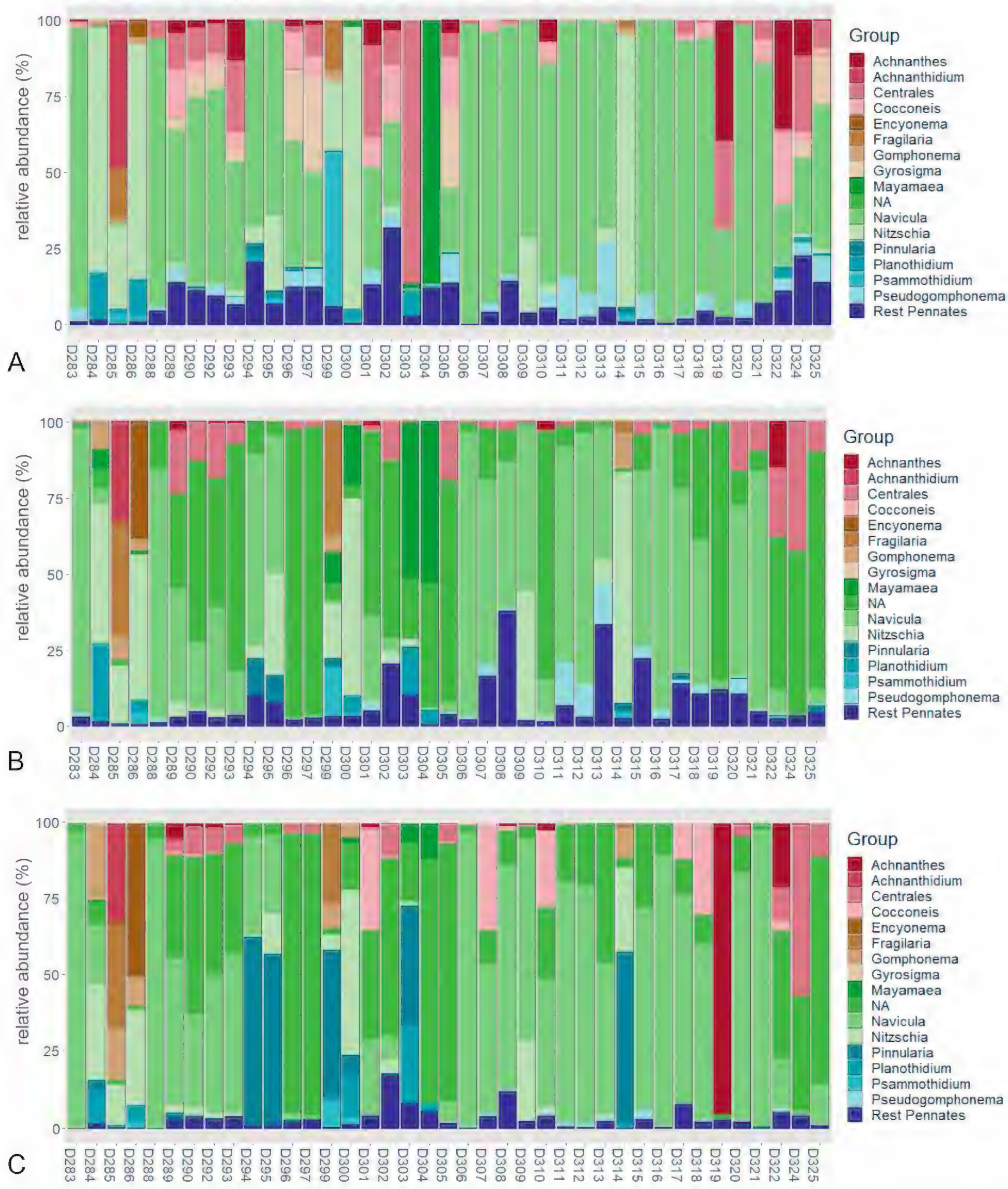

Figure 9. Relative abundance (%) of diatom genera across all sample locations. **A** Morphology. **B** *rbc*L marker gene. **C** 18SV4 marker gene.

assigned genera, the morphology inventory shows a high abundance of *Navicula* (D289–D293, D296, D297, D310). A comparison between metabarcoding and the morphology inventory shows that some genera were disproportionately higher in relative abundance like *Encyonema* in sample D286. This tendency of overrepresentation was more evident in the 18SV4 inventory, e.g. in the case of *Pinnularia* in most of the freshwater and brackish water samples and *Achnanthes* in sample D319. Interestingly, the genus *Cocconeis* was almost absent in the *rbc*L dataset.

Community analysis

Average taxa richness across water and substratum type was always higher in the metabarcoding inventories than in LM (Table 5, Suppl. material 1: table S1: results for the single sample locations). The Shannon diversity index based on the relative abundance of taxa was higher in the metabarcoding inventories as well (Table 5). The average diversity obtained for LM, *rbc*L and 18SV4 were 1.46, 1.94 and 1.85 respectively. The three approaches agree that a low diversity was found in marine habitats in biofilm from rocks and the highest diversity in marine or brackish waters in epipsammic biofilms.

The NMDS plots for morphology, *rbc*L and 18SV4 inventories show a clear separation in the community composition of samples taken from marine and freshwater habitats (Fig. 10, stress =0.1). Brackish water habitats are more similar to freshwater habitats. Among marine habitats, community composition is more similar among samples taken from the same substrate. An exception is found in the LM dataset (Fig. 10C). Here D301 and D310 although taken from biofilm of stones are more similar to samples taken from epipsammic locations. The distinct separation was confirmed by PERMANOVA. Statistically significant differences in the community composition were found for the LM and DNA inventories among different water types (LM: $F_{2,36}$ = 8.588, p = 0.001; *rbc*L: $F_{2,36}$ = 4.454, p = 0.001; 18SV4: $F_{2,36}$ = 6.316, p = 0.001) and substratum types (LM: $F_{1,37}$ = 8.899, p = 0.001; *rbc*L: $F_{1,37}$ = 6.853, p = 0.001; 18SV4: $F_{1,37}$ = 7.309, p = 0.001).

According to the SIMPER results (Suppl. material 1: table S2), the species or ASVs contributing the most to the dissimilarity regarding the water types were *Navicula* cf. *perminuta* (marine–freshwater: LM 26.0%, 18SV4: 13.2%), *Mayamaea sweetloveana* (marine–freshwater: rbcL: 7.8%), *Navicula gregaria*

Table 5. Average taxa Richness and Shannon diversity index across water and substratum types with the morphological and DNA metabarcoding inventories (*rbc*L and 18SV4).

	LM		*rbc*L		18SV4	
	Taxa richness	**Shannon index**	**Taxa richness**	**Shannon index**	**Taxa richness**	**Shannon index**
Marine, biofilm from stones	10	0.8	58	1.9	39	1.3
Freshwater, biofilm from stones	12	1.1	42	1.8	58	2.1
Marine, epipsammic biofilm	43	2.8	73	2.0	164	2.5
Brackish water, epipsammic biofilm	16	1.6	40	2.2	80	1.9

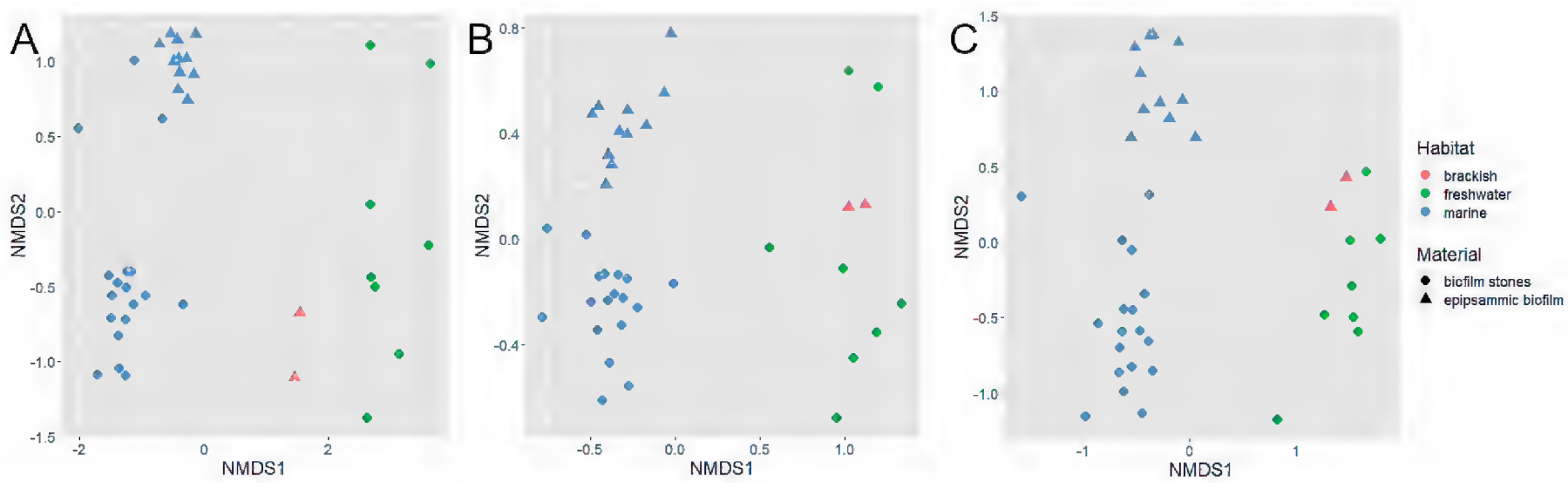

Figure 10. NMDS multivariate clustering of benthic diatom communities regarding water type and substratum type. **A** Morphology. **B** *rbc*L marker gene. **C** 18SV4 marker gene. Stress: 0.1 (**A–C**).

(marine–brackish water: LM: 26.2%, *rbc*L: 16.0%; freshwater–brackish water: LM: 26.8%, *rbc*L: 14.5%) and *Pinnularia australomicrostauron* (marine–brackish water: 18SV4: 29.3%; freshwater–brackish water: 28.7%). The dissimilarities regarding the substrate type were influenced by *Navicula* cf. *perminuta* (LM: 27.9%) and two ASVs, that could not be taxonomically assigned (*rbc*L: 12.0%, 18SV4: 22.7%).

Discussion

Benthic diatom diversity in Potter Cove, Antarctic Peninsula

This study demonstrated that the shallow coastal zone of Potter Cove harbours a rich diatom community with a total of 116 marine taxa identified by morphological investigation. Two floristic studies on benthic diatoms were already performed in Potter Cove by Al–Handal et al. (2022) and Al–Handal and Wulff (2008a), which retrieved 80 and 84 taxa respectively. However, here only surface sediments at four locations were sampled by SCUBA diving in different depths. A comparable study on neighbouring Livingston Island (Zidarova et al. 2022) with a larger variety of sampling locations also found a higher number of 133 taxa.

Even though fewer freshwater samples in our study were evaluated, 93 taxa were still found in these habitats. In general, many more studies investigating freshwater rather than marine habitats in Antarctica have been performed to date. Floristic studies found 122 taxa on King George Island/Isla 25 de Mayo (Kochman–Kędziora et al. 2018), 102 taxa on Livingston Island (Sterken et al. 2015) and 69 taxa on James Ross Island (Kopalová et al. 2012). Numerous new species endemic to maritime Antarctica have been described in recent decades e.g. Van de Vijver et al. (2006); Zidarova et al. (2009); Kopalová et al. (2011); Van de Vijver et al. (2012); Van de Vijver et al. (2013a); Kopalová et al. (2015); Zidarova et al. (2016b) and it is estimated that 44% of all species might be endemic to the Antarctic, and most of them are confined to a single biogeographic region (Verleyen et al. 2021).

This study demonstrated that DNA metabarcoding presents an efficient method for surveying diatom biodiversity in coastal and freshwater ecosystems as it recorded a similar number of genera as the LM method with a high proportion of the genera identified by both methods. However, there are some discrepancies between the inventories. Some genera and species (23 and 73, respectively) were exclusively identified by DNA metabarcoding. DNA metabarcoding based on both marker genes retrieved a higher number of ASVs than taxa identified by LM. Several ASVs, however, were then assigned to the same taxon by the metabarcoding pipeline. Due to the incompleteness of the reference library the number of assigned species was lower for both marker genes in the metabarcoding approach compared to the LM approach which showed a greater efficiency for identifying taxa at species level.

Despite those restraints, similarity analyses of morphological as well as molecular data led to the same results. There was a clear statistically significant separation of diatom community according to water and substratum type. Based on all three approaches marine communities differ from freshwater communities and the brackish water communities are more similar to the freshwater ones. In addition, substratum type (sand or stones) seems to be a factor leading to dissimilarities in the diatom community as well. However,

species contributing most to the dissimilarities between habitats differed, due to discrepancies in the inventories, which are discussed later.

Importance of the taxonomic reference library

60 diatom species were cultured and helped assign 47 taxa in our metabarcoding dataset because their sequence data were new to science. In the case of 27 taxa, sequence data was uploaded to ENA or GenBank in this or previous studies analysing the data from the same sampling campaign. Taxa, where a taxonomic investigation is still needed, will be published in combination with their sequence data, when a thorough taxonomic treatment is completed. Many of them will probably be described as new. In advance, their data is available at the Herbarium Berolinense. The large fraction of unidentified taxa especially in the marine habitat (~68%) is not surprising since benthic diatoms were not broadly studied in this habitat.

Interestingly, some taxa established in culture were not observed in the morphological inventory. This was already shown in Mexican and Canadian streams in Mora et al. (2019) and Skibbe et al. (2022) where culture media and culturing conditions (i.e., light, day/night cycle and temperature) were listed as possibilities for the concealed diversity revealed by clonal culturing. Those may have allowed taxa to grow that were otherwise too rare to be detected through microscopy examinations. Valves of *Lunella* sp., which were available due to culturing in this study, are very small, only lightly silicified with no visible ornamentation in LM. Valves might have been mistaken with non–diatom material or destroyed in processing of the samples as treatment with Naphrax tend to destroy delicate valves (Vermeulen et al. 2012). The living cells of this species in enrichment medium might have been easier to spot due to their chloroplasts.

The multitude of successfully grown taxa indicates that our approach using several culture media with different salinities was suitable for culturing benthic diatoms from Potter Cove. Even though an extensive culturing effort was undertaken, many taxa could not be established as a unialgal culture. They were not observed as living cells in our enrichment culture as they might not be sampled alive, culture conditions were not suitable, or long–distance shipment might have destroyed more delicate species. Furthermore, some taxa were not able to grow after single cell isolation or the unialgal culture died before enough material was available for analysis. Therefore, an increased diversity of culture media and variation of culture conditions (e.g., temperature, agitation, light intensity or day/night cycle) could potentially stimulate the growth of additional less competitive species and thus improve culture success.

In our metabarcoding dataset many of the taxa could not be assigned by the reference library, even on genus level. This is especially true for marine habitats. The reference library established from the sequence database from the Herbarium Berolinense comprises mostly freshwater diatoms and already Pérez–Burillo et al. (2022) showed that the data availability in the Diat.barcode reference library has a strong tendency towards freshwater species. However, recent metabarcoding studies conducted in freshwater habitats highlight the need for a comprehensive reference database as well e.g. Rivera et al. (2018); Mortágua et al. (2019); Kulaš et al. (2022) to improve metabarcoding in routine monitoring.

Rimet et al. (2018a) suggested to complete reference libraries by using metabarcoding data. This could be a promising tool, however, the sequence needs

to be abundant in the sample, with no insertions or deletions or stop codon and phylogenetic neighbour taxa have to correspond to neighbour taxonomic taxa expected from morphological observations. For taxa not matching those criteria, unialgal cultures as a reference for DNA metabarcoding studies are still needed. Furthermore, established data through culturing supports an integrative taxonomy as cultures show morphological variability within a species (Mohamad et al. 2022). In addition, sequence data supports phylogenetic analyses of diatoms (Kociolek et al. 2013; Downey et al. 2021) and especially longer sequences than short metabarcodes are needed for defining deep nodes of classification trees (Rimet et al. 2018b).

Discrepancies between morphological and molecular results

Several discrepancies between the morphological and the molecular inventory were evident. Most obvious was the above discussed fact, that many species and some genera were not encountered in the molecular inventory since the reference database was lacking a representative sequence. This was the case for e.g. *Gyrosigma* sp., *Pteroncola carlinii* Almandoz & Ferrario or *Achnanthidium* cf. *maritimo–antarcticum* listed with a relative high abundance in the LM inventory but without an entry for both metabarcoding inventories since both barcode sequences are unknown. Furthermore, some samples, where a high abundance of taxa in LM identified to the genera like *Navicula* and *Gyrosigma*, had no corresponding match in the metabarcoding inventories. This is indeed surprising, since those genera have a rather intensive representation in the reference databases. Studies in the last decades have shown that taxa morphologically assigned to an existing genus in Antarctica had been actually force fitted. Several new genera in the Antarctic or southern hemisphere have been established and existing taxa underwent a new combination (Williams 1988; Bishop et al. 2019; Guerrero et al. 2021). Our dataset suggests that there is a high cryptic diversity, which highlights the need for intensive taxonomic investigation of benthic diatoms in this region. An additional reason for discrepancies between the inventories might be found in morphological destruction or overlooked valves might lead to underrepresentation of taxa in LM like in the above described case of *Lunella* sp.

One of the key issues concerning sediment DNA metabarcoding is the distinction of living organisms that are part of the active benthic community from those organisms that are represented either by inactive resting stages or solely by DNA traces (Pawlowski et al. 2022). Sediments act as a repository of both intra– and extracellular DNA and the presence of extracellular DNA may have also influenced our molecular inventory, since taxa might have been detected in a sample even if their cells are not physically present. Those factors make it difficult to differentiate between living and dead organisms, or between species that live in the sediments or that have been settled from the water column.

Varying gene copy numbers per organism due to cell size and number of chloroplasts per cell is probably another reason for discrepancies between the LM and metabarcoding inventory. This correlation was noted in the case of *rbc*L by Vasselon et al. (2018) and Pérez–Burillo et al. (2022) and in the case of 18SV4 by Mora et al. (2019). This likely explains the higher abundances obtained by the DNA metabarcoding for the big cell species *P. australomicrostauron* and *Paralia sol* (≡*Ellerbeckia sol*) in our study, which was especially apparent in the 18SV4 dataset.

The poor representation of *Cocconeis* in the *rbc*L inventory (1025 reads, 2 ASVs) despite the very high diversity of *Cocconeis* species revealed by LM was also an issue in the study of Burillo et al. 2022. Sequences of the Antarctic species *C. fasciolata* were available in our reference database as a culture of this species was established. No ASVs were assigned to this taxon in the *rbc*L inventory in contrast to the 18SV4 inventory. A worrying possibility is that primers of the *rbc*L barcode might not be suitable for marine *Cocconeis*. In fact, in comparable freshwater studies *C. placentula* was the most abundant taxon (Vasselon et al. 2017; Kulaš et al. 2022). A comparison with the forward *rbc*L primer region with the sequence of *C. fasciolata* showed a transition from the base G to an A. We suggest here to include a modified Diat_*rbc*L_708F forward primer at the third position from the back in the primer mix for marine samples: TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GAGGTGAAACTAAAGGTTCWTACTT**R**AA.

In general, the list of taxa with the highest relative abundance of the LM data set correlates better with the *rbc*L than with the 18SV4 inventory. Similar results were found by Bailet et al. (2019), where the use of the 18SV4 marker generated more species inventories discrepancies. Bailet et al. (2020) investigated the performance of the *rbc*L and the 18SV4 marker using different bioinformatic pipelines. Here in addition, the use of the *rbc*L marker resulted in outcomes closer to these generated using traditional microscopy. Furthermore, it was shown that the choice of the pipeline had an influence on the taxonomic assemblage, but the results generated by *rbc*L correlated better among pipelines.

Prospects of DNA Metabarcoding for Antarctic benthic diatoms

It has been shown that the metabarcoding approach can complement and improve traditional identification via LM. It enables to detect tiny and delicate species. *Lunella* sp. and *Cylindrotheca* cf. *closterium* were detected in metabarcoding but not via the count of valves in LM. Rare species may be detected as well. In traditional identification, generally a few hundred valves are counted per sample probably not reaching saturation of species richness, while in metabarcoding several 10,000 to 100,000 reads are usually evaluated. Furthermore, it may detect cryptic diversity. Species that are morphologically similar may be better separated in the metabarcoding dataset.

In addition to the extension of information about Antarctic diatom diversity, our study also provided a new tool to survey water quality changes in Antarctica. In recent decades, climate change has had a crucial impact in the polar regions with increasing air and water temperature leading to glacial melting and the accompanying freshwater increase in coastal areas (IPCC 2019). DNA metabarcoding evaluation with a continuous sampling routine can give a valuable insight on community changes of benthic diatoms. Using those microorganisms as bioindicators may help assess the biological status and quality changes of water bodies in Antarctica, where environmental conditions are quickly evolving.

Conclusion

Antarctica is among the most extreme environments on Earth. An increased research effort is required in the light of desynchrony between the pace of

change in polar regions and information demands to face engendered challenges (Danis et al. 2020). This study showed that a high benthic diatom diversity is apparent in this region, which was shown by traditional morphological identification and by the DNA metabarcoding approach. Overall, a combination of morphological, metabarcoding approaches accompanied by culturing increases the detection and identification of diatoms as the methods provide complementary information on biodiversity of benthic diatoms in this region. Furthermore, culturing is needed to enrich the reference barcode database. Ultimately, diatom diversity based on three approaches allowed a reliable dataset that can be used in routine monitoring assessment, which provides a deeper understanding of ecological status. Many taxa in both approaches could still not be identified on species level which emphasises the need for further taxonomic investigations in this region. In addition, the need for more efforts to complement the taxonomically curated reference database is evident.

The slides of the environmental samples, morphological and molecular data gained by LM and SEM investigation as well as sequencing of cultures together with the metabarcoding dataset represents the currently most extensive biodiversity dataset of marine benthic diatoms of Western Antarctica. All voucher material as well as the data are deposited at the Herbarium Berolinense and could be used as a baseline for further investigations, as a reference for monitoring routines and as training records in modelling tasks.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

KS and JZ developed the concept of this study. JZ and GC sampled and OS isolated, purified and established clonal cultures. KS, HM and JZ performed the metabarcoding analysis. KS provided the light microscopic analysis and KS, NA and RJ did the taxonomic identification. NA and WHK are responsible for the curation and data curation. KS wrote the first version of the paper. All authors edited and approved the final version of this manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information. Raw demultiplexed reads were deposited at GenBanks Sequence Read Archive and are publicly available under project number PRJNA997374.

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Supplementary material 1

Statistic results

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Explanation note: **table S1.** Taxa Richness and Shannon diversity of the sample sites with the morphological and DNA metabarcoding inventories (*rbc*L and 18SV4). **table S2.** SIMPER results listing the four most contributing species or ASV's to the dissimilarities between samples taken from different water types (freshwater, brackish water and marine) and substratum types (epipsammic biofilm, biofilm on rocks) for the LM, the *rbc*L and the 18SV4 inventories, CC: Cumulative contribution to dissimilarity, AA: Average abundance across all samples.

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